"IDENTIFICATION OF POTENTIAL GENETIC RISK MARKERS IN FAMILIAL PRIMARY OPEN-ANGLE GLAUCOMA IN TASMANIA"

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This thesis is submitted in fulfilment of the requirements for the degree of Master of Medical Science (Ophthalmology)

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This thesis contains only material which is original, except where due acknowledgement is given.

This work has not been submitted or accepted for the award of any other degree or diploma in any other university or educational institution.

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Johnny Wu

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LIST OF ABBREVIATIONS

A & V	- central retinal Artery and Vein
CI	- Confidence Interval
GIST	- Glaucoma Inheritance Study in Tasmania
GLC1A	- Glaucoma 1A locus
IOP	- IntraOcular Pressure
JOAG	- Juvenile-onset Open-Angle Glaucoma
KD	- KiloDalton
LC	- Lamina Cribrosa
MYOC	- Myocilin
N	- Number of cases

NOS - Nitric Oxide Synthase

OAG - Open-Angle Glaucoma

OH - Ocular Hypertension

OR - Odds Ratio

P - Probability

POAG - Primary Open-Angle Glaucoma

SAS - SubArachnoid Space

SSCP - Single-Strand Conformation Polymorphism

TIGR - Trabecular Meshwork-Induced Glucocorticoid Response

USA - United States of America

ACKNOWLEDGEMENTS

"The direction in which education starts a man,

will determine his future life."

As Thomas Edison once said, "genius is 1% aspiration and 99% perspiration". This project has been made possible only with the collaborative help and support of many people over the past two years, including volunteer ophthalmologists, optometrists, nurses, orthoptists and medical students who helped examine extended families all over Tasmania.

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ABSTRACT

Approximately 50% of all primary open-angle glaucoma (POAG) is familial. Investigation of clinical risk factors associated with familial POAG may help to identify phenotypic subtypes of the disease, each with different pathophysiologic mechanisms that may be modified by intervention and disease-prevention strategies.

In a cross-sectional retrospective study of 2940 'glaucoma' patients over 10 years of age in Tasmania, the prevalence of nine potential clinical risk factors (hypertension, diabetes mellitus, migraine headache, corticosteroids use, smoking, atherosclerosis, cold extremities, blood transfusion, thyroid disorders) were compared using multi-stepped regression analysis between 1014 patients with familial glaucoma and 688 patients with sporadic or non-familial glaucoma classified by Glaucoma Inheritance Study in Tasmania (GIST) scores (intraocular pressure, optic disc and visual field changes) and genealogic data. 59.6% of all subjects with POAG have a positive family history of POAG.

There is no significant difference in the distribution of gender between the familial and sporadic POAG groups (OR 1.053; 95%CI 0.819-1.353).

No risk factor examined is significantly different in the familial glaucoma group in comparison to the sporadic glaucoma group, after adjusting for confounding effects of age and gender, GIST scores, degree of relatives or other potential clinical risk factors.

The distribution of GIST scores in the familial glaucoma group is skewed towards the higher spectrum and is significantly different from that of the sporadic glaucoma group ($p<0.001$), suggesting the likelihood of an earlier onset and/or a greater severity of glaucoma in the familial group.

CHAPTER I

INTRODUCTION

"It is amazing how such a small window can open out to such a large world."

(William Shakespeare)

I.I *GLAUCOMA*

After cataract, glaucoma is the second leading cause of blindness in the world, with an estimated worldwide prevalence of approximately 70 million people, half of whom have open-angle glaucoma (Flanagan 1993; Shields 1982; Spaeth 1998; Quigley 1996). Infrequent in people below the age of 50, glaucoma affects up to 10% of the population over the age of 80 (Wensor 1998).

Currently, glaucoma is not considered to be a disease *per se* but rather a slowly progressive, insidious optic neuropathy characterised by a specific pattern of optic nerve damage, which includes optic disc excavation ('cupping'). The subsequent visual field loss may represent the endpoint of a final common pathway resulting from a number of different aetiological agents affecting the eye (Vaughan 1992; Berson 1993; Drance 1988; Horton 1998).

At present, there is no known treatment to restore vision lost from glaucoma. However, early detection of the disease and administration of appropriate treatment can prevent blindness (Hoskins and Kass 1989). Visual impairment resulting from glaucoma imposes a significant physical, emotional, social and economic burden on an individual and has a negative impact on quality of life (Weih 2000).

Approximately 50% of all glaucoma is familial (Mitchell 1996; McNaught 2000). Many studies have examined the relationship between total (familial and sporadic) primary open-angle glaucoma (POAG) and various clinical risk factors, such as hypertension, diabetes mellitus and migraine, but no study to date has yet compared the relationship between these risk factors and the familial or sporadic glaucoma subgroups. The aim of this thesis is to investigate clinical risk factors associated with familial POAG; these risk factors may be used to identify phenotypic subtypes of the disease, each with potentially different pathophysiologic mechanisms that may be modified by intervention and disease-prevention strategies.

I.II *CLASSIFICATION*

In terms of pathogenesis and clinical expression, glaucoma is a highly heterogeneous group of eye disorders (Spaeth 1998). It has a few generally accepted and clinically valid subgroups (Caprioli 1993), including those based on anterior chamber width, age of onset, intraocular pressure (IOP) level and disease progression (Spaeth 1976; Geijssen 1987).

As illustrated in **Table I.1**, glaucoma can be classified into three broad categories based on the cause for poor aqueous outflow: Open-angle, Closed-angle and Congenital/Developmental. Congenital glaucoma presents at birth or during the first year of life with buphthalmos, and may be associated with an inherited syndrome of ocular anterior segment dysgenesis (Craig 1999). Each of these can be further subdivided into primary types in which the glaucoma is not associated with any other systemic or ocular disorder and secondary types resulting from a pre-existing ocular or systemic disease (Duke-Elder 1941). Indeed, recognition of the spectrum of manifestations of glaucoma is required for early diagnosis and appropriate management of individual cases.

Table I.1 A classification system for glaucoma (Vaughan 1992; Alward 1998).

Types	Open-angle	Closed-angle	Congenital/ Developmental
Primary	**POAG** **JOAG** **low-tension glaucoma**	**pupillary block** **plateau iris syndrome**	**Primary** **associated with** ***aniridia** ***Axenfeld-Rieger syndrome** ***neurofibromatosis** ***Peter's anomaly** ***Sturge-Weber syndrome**
Secondary	**angle recession** **aphakia** **corticosteroid** **pseudoexfoliation syndrome** **ghost cells** **haemmorrhage&** **neuovascularisation** **inflammation & uveitis** **phagolytic changes in the iris** **pigment dispersion syndrome**	**Aqueous misdirection (malignant glaucoma)** **ciliary body swelling** **ectopic lentis** **epithelial downgrowth** **Fuch's endothelial syndrome** **iridoschisis** **posterio polymorphos** **corneal dystrophy**	**aphakia** **retinoblastome** **retinopathy of prematurity**

JOAG – Juvenile-onset open-angle glaucoma; **POAG** – Primary open-angle glaucoma.

I.III *PRIMARY OPEN-ANGLE GLAUCOMA (POAG)*

Open-angle glaucoma is the most common type of glaucoma in 'Western' developed countries (Flanagan 1993). The iridocorneal angle is not narrowed, but the aqueous humour that flows through the pupil into the anterior chamber cannot pass through the trabecular meshwork into the normal venous drainage system (**Figure I.1a**). This is usually secondary to an increased resistance to aqueous flow in Schlemm's canal or in the trabecular meshwork (**Figure I.1b**) (Vaughan 1992; Berson 1993). Most open-angle glaucoma is of the primary type, but it can also be congenital or secondary due to other abnormalities such as pseudoexfoliation syndrome, neovascularisation, uveitis, and pigment dispersion syndrome (Alward 1998).

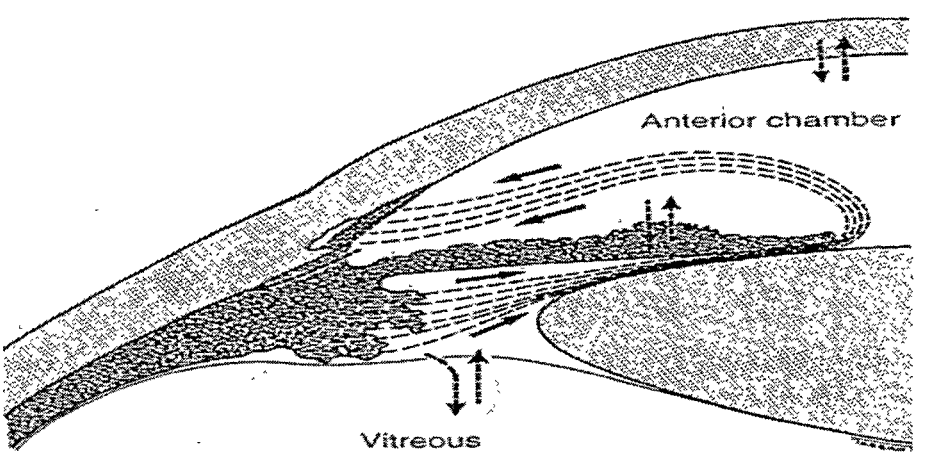


Figure I.1a. Schematic diagram of the anterior segment of the eye. The conventional pathway followed by aqueous humour from the posterior chamber to the anterior chamber and outflow pathways are shown by arrows (Bron et al, 1997 pp302).

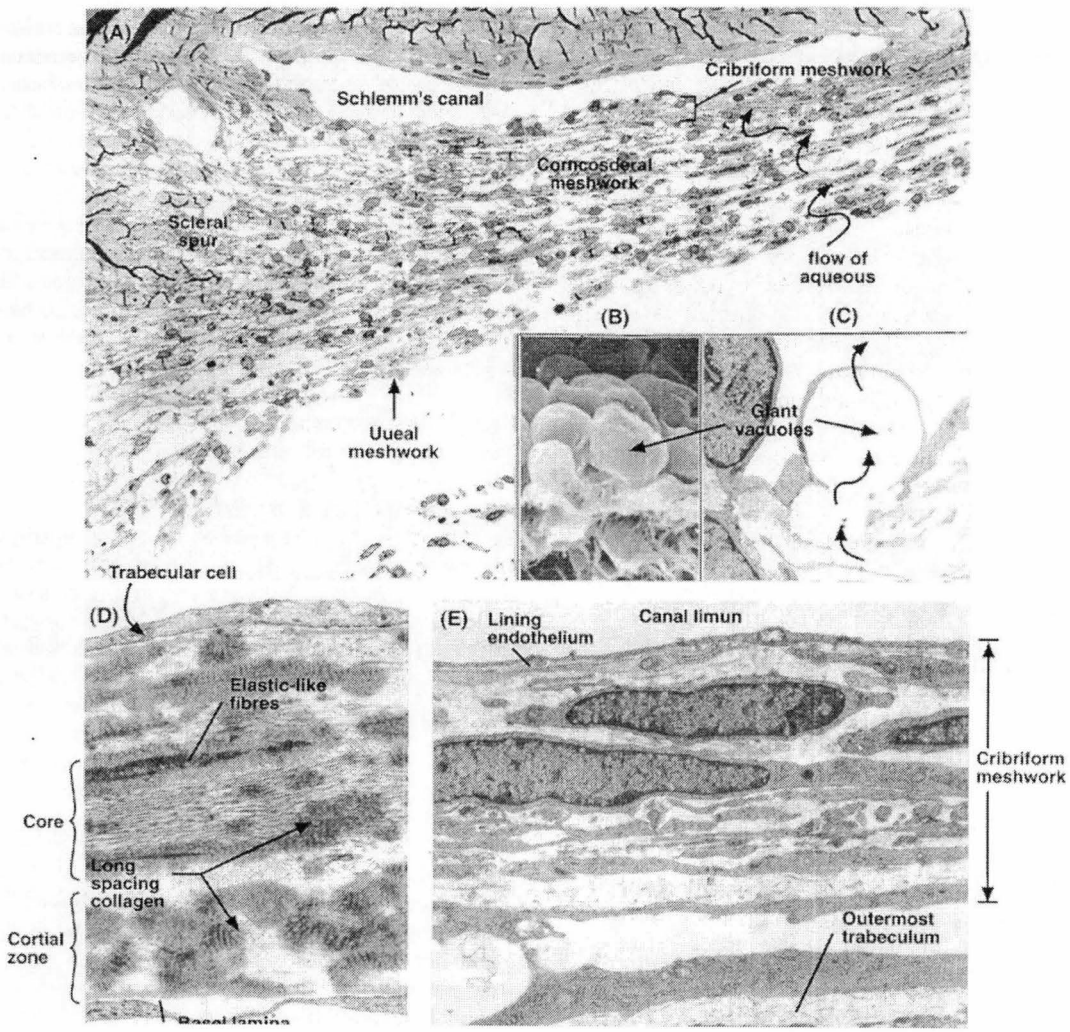


Figure I.1b. Histology and ultrastructure of the trabecular meshwork and Schlemm's canal (Forrester et al, 1999 pp21).

POAG, also known as chronic simple glaucoma, is generally a bilateral although not necessarily symmetrical disease, often with the following characteristics:

- Adult onset (usually in the sixth decade)
- IOP greater than 21 mmHg at some point in the course of disease
- Open anterior chamber angle of normal appearance with visualisation of the full extent of trabecular meshwork, scleral spur and iris processes on gonioscopy (Vaughan 1992)
- Glaucomatous optic nerve head damage, such as optic disc enlargement associated with disc pallor in the area of cupping, attenuated neural rim with dipping of retinal vessels (bean pot cup), cup-disc ratio > 0.5 or significant asymmetry between the two eyes (cup-disc ratio difference > 0.2), splinter haemorrhages and atrophy of the nerve fibre layer (Hoyt's sign) (**Figure I.2**) (Sommer 1991; Vaughan 1992; Tuulonen 1993)
- Visual field loss, such as the characteristic Bjernum scotoma, arcuate scotoma and nasal step of Roenne (**Figures I.3a; I.3b; I.3c**) (Vaughan 1992; Kanski 1999).

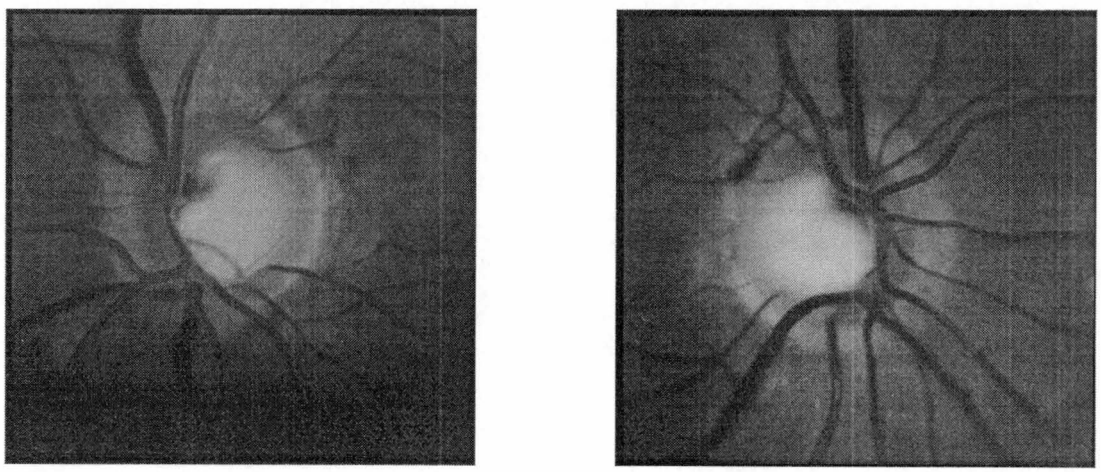


Figure I.2. Photographs of glaucomatous optic nerve head damage

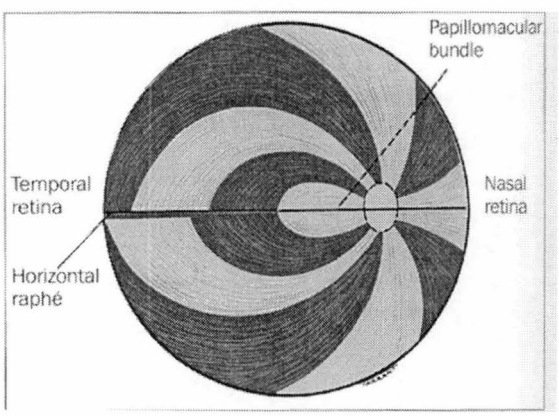


Figure I.3a Anatomy of the retinal nerve fibers (Kanski, 1999 pp194).

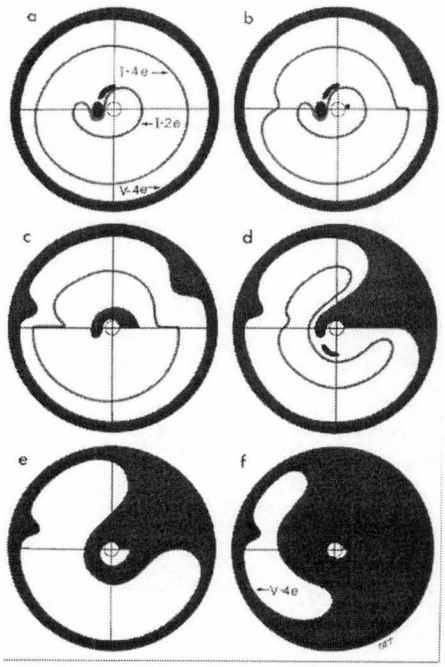
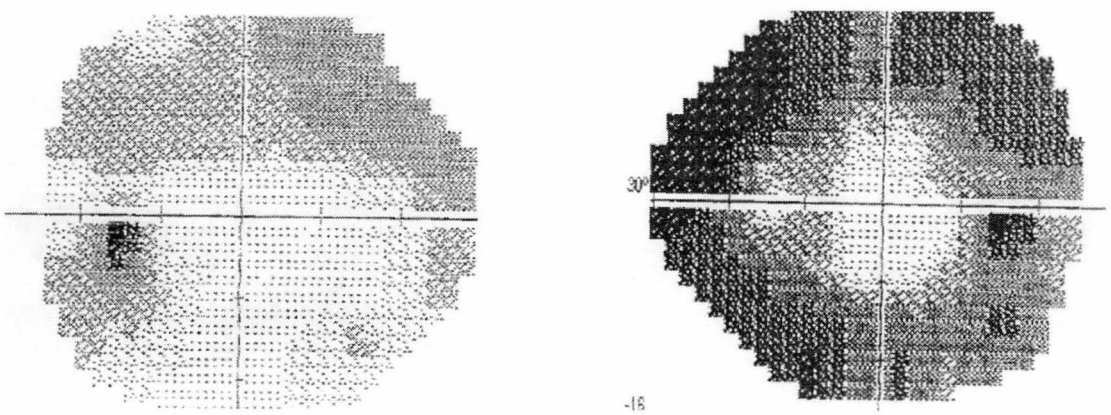


Figure I.3b Progression of the visual field loss (Kanski, 1999 pp204).



Left eye

Right eye

Figure I.3c Visual field analysis of an affected glaucoma patient from the study.

It must be noted, however, that there is a lack of universal agreement on the definition and hence diagnosis of glaucoma (Kahn 1980; Wilson 1993).

Most people who develop POAG notice no symptoms until vision is impaired and damage is marked, giving it the reputation as the "sneak thief of sight" (Vaughan 1992; Berson 1993). In developed countries, up to 50%-60% of those affected by glaucoma remain undiagnosed (Mitchell 1996; Quigley 1996; Wensor 1998; Weih 2001). As optic nerve fibres are damaged by glaucoma, scotomas may begin to develop insidiously over many years, initially affecting peripheral nasal vision that is well covered by the field of the fellow eye (Vaughan 1992; Berson 1993; Spaeth 1998; Kanski 1999). In the later stages of the disease peripheral temporal and central 3-10 degrees of vision are affected (Vaughan 1992; Berson 1993; Spaeth 1998; Kanski 1999).

Approximately 16% of all patients with otherwise characteristic POAG have IOPs consistently under 22mmHg and they form a subgroup referred to as "normal-tension" or "low-tension" glaucoma (Kanski, 1999).

Juvenile-onset open-angle glaucoma (JOAG) is an uncommon subtype, characterised by a more severe course and an earlier onset (typically <35 years of age although the cutoff age is not consistent between authors) (Kitsos et al. 1995).

I.IV *PATHOPHYSIOLOGY*

It is generally accepted that increased resistance to aqueous outflow in the drainage channels causes elevation of IOP in POAG and that the development of visual field loss is related to progressive loss of axons in the optic nerve head. However, speculation abounds as to how the two processes are linked. Several mechanisms have been postulated to explain the optic nerve head damage, but no single mechanism can adequately explain the great variation in susceptibility to damage and the pattern of damage seen. Drance (1988), proposed at least two mechanisms of glaucomatous optic nerve injury:

1) The **Ischaemic** theory postulates that compromise of the microvasculature of the axons in the optic nerve head plays a role (**Figure I.4a; I.4b**), possibly via

a) loss of capillaries

b) alteration in capillary blood flow

c) blood vessel structural changes that disturb the delivery of nutrients or removal of wastes and metabolic products from the axons

d) dysregulation of blood flow and/or

e) delivery of damaging vasoactive substances to the blood vessels of the optic nerve head (Kanski 1999).

This 'pressure-independent' mechanism could be responsible for localised visual function loss, with chronic ischaemia a possible risk factor. Moreover, ocular vasospasm may cause recurrent ischaemic damage to the optic nerve (Drance 1988).

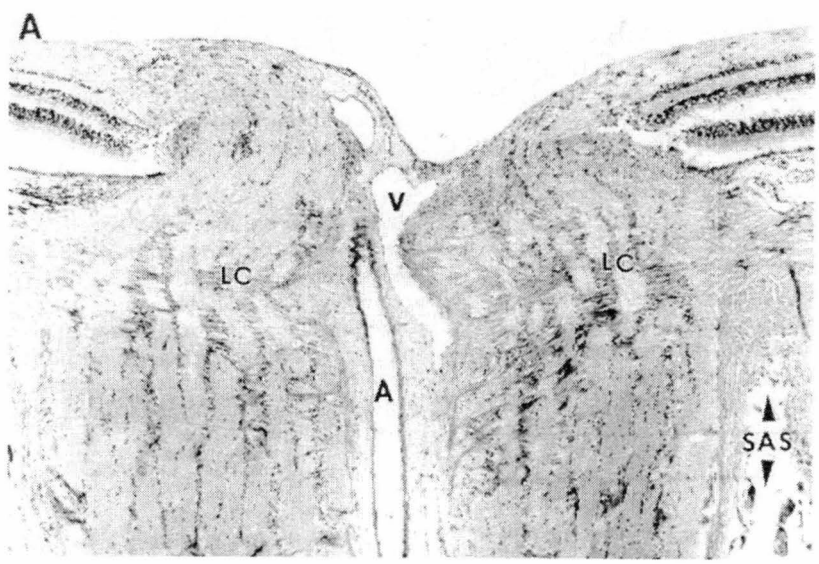


Figure I.4a. Histology section of the optic nerve head. LC, lamina cribosa; A&V, central retinal artery and vein; SAS, subarachnoid space (Forrester et al, 1999 pp48).

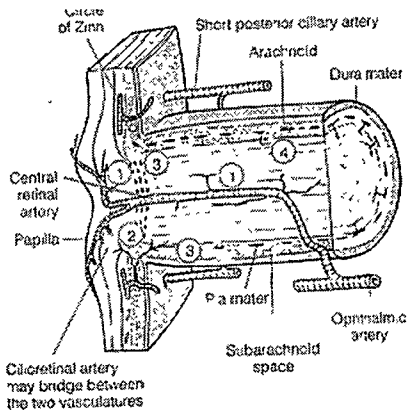


Figure I.4b Blood supply of the optic nerve (Forrester et al, 1999 pp48).

2) The Direct **Mechanical** theory suggests that chronically elevated IOP directly damages the ganglion cell axons as they pass through the lamina cribrosa by inducing collapse or deformation of the lamina plates (**Figure I.4c**). This can impinge directly on nerve fibres and compress vasculatures, resulting in inadequate delivery of nutrients to the axons in the optic nerve head (Kanski 1999). Another possibility is apoptotic death of retinal ganglion cells, associated with IOP via a yet unidentified cellular pathway (Wiggs 2000). This 'pressure-dependent' mechanism could account for diffuse visual function loss, with elevated IOP as the main risk factor (Drance 1988).

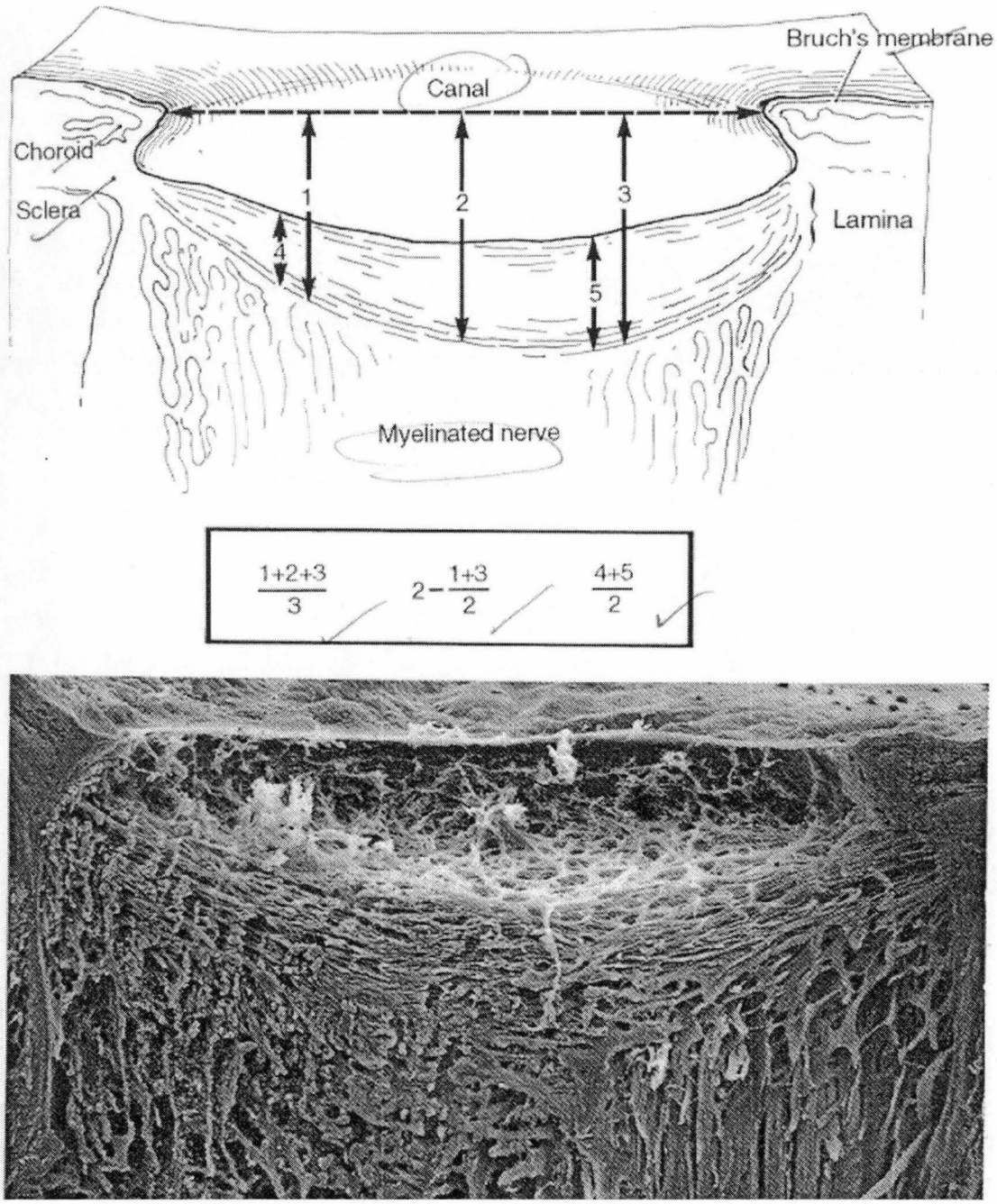


Figure I.4c. Sectional view of optic nerve head in glaucoma (Bron et al, 1997 pp497).

I.V *GLAUCOMA INHERITANCE & FAMILY HISTORY*

"Hereditary glaucoma" was first reported by von Graefe in 1869. In 1941, Duke-Elder (1941) described "familial glaucoma", a type of glaucoma that was inherited in a dominant manner. To date, many studies have examined familial aggregation, inheritance and modes of transmission in selected families across the world (Brezin 1997; Litcher 1997; Angius 1998).

Cross-sectional epidemiological studies have shown that 45-55% of POAG patients report a positive family history of glaucoma (Mitchell 1996; McNaught 2000; Williams-Lyn 2000), which is a risk factor for progression of ocular hypertension into POAG (Tielsch 1994; Nemesure 1996; Quigley 1994; Rosenthal 1985; Leske 1996). Early evidence of glaucoma inheritance was observed in twin studies on monozygotic twins, which demonstrated concordance for POAG, although there have been exceptions (Teikari 1987). Concordance was higher between identical twins compared with fraternal twins, but the estimated genetic association was relatively weak in many cases, indicating a potential role for additional nongenetic or environmental factors (Armaly 1967).

Many pedigrees of POAG have since been studied, but only some seem to follow simple Mendelian autosomal dominant transmission through several generations (Frey and Posner, 1952; Francois 1961). Statistically, 50% of all offspring of an index case should be affected, but the prevalence is often found to be much lower in POAG, around the order of 10% (Wolfs 1998). Incomplete penetrance and a subtle phenotype have been suggested as a possible explanation (Jay and Patterson 1970). Furthermore, autosomal recessive inheritance

patterns have also been suggested, particularly from the identification of corticosteriod-induced ocular hypertension in 1963 (Becker and Mills 1963) and from advances in understanding of primary congenital glaucoma (Gencik 1998). The simple Mendelian modes of inheritance cannot adequately explain transmission in many cases of POAG, and there is growing support for a multifactorial model in which multiple genes act together with certain unidentified environmental factors (Jay and Patterson 1970; Merin and Morin 1972). These varied findings support the premise that POAG comprises a spectrum of diseases rather than one clinical entity, with different forms displaying varying modes of inheritance.

The results of the various family studies on POAG are difficult to compare because of differences in method of case ascertainment, definition of glaucoma, age criteria for participation, and number of years of follow-up. Often, only a family history was taken into account, or a limited number of family members were examined. These studies relied almost exclusively on observational data and used anamnestic recollections of POAG status in relatives to estimate heritability, thereby introducing selection bias (Nguyen 2000).

Studies using multivariate analytic techniques have added supportive evidence for the role of family history as a significant risk factor (Wilson 1987; Drance 1973), estimating that a positive family history of glaucoma gives a 3-fold increase in risk of developing POAG (Leske 1996; Leske 1983; Leske 1995; Tielsch 1994). The Blue Mountains Eye study in Australia, which utitised a multivariate model and adjusted for age, gender, and other variables, also found that a first-degree family history of glaucoma is significantly associated with a previous diagnosis of open-angle glaucoma (OAG) or ocular hypertension (OH) (OR 3.6; CI 1.8-7.2) (Hourihan 1999). Similarly, the Visual Impairment Project found positive

family history to be the strongest risk factor for glaucoma after adjusting for age (OR 3.1; 95%CI 1.6-5.3)(Weih 2001).

There is an increased prevalence of POAG among first-degree relatives of patients with the disease, with as many as 2.8% to 13.5% being affected compared to a prevalence of 0.5% to 2% in the general population (Perkins 1974; Francois 1966; Becker 1960; Shaffer 1965; Nguyen 2000).

In a Dutch population-based study in Rotterdam, glaucoma status was ascertained by visual field defects and cup-disc ratios of 0.7 or higher or asymmetry of 0.3 or higher between both eyes (Wolfs 1998). The prevalence of glaucoma was 10.4% in siblings of patients compared with 0.7% in siblings of controls; it was 1.1% in offspring of patients compared to 0% in offspring of controls. That is, a person's risk of POAG was increased by a factor of 2 if a parent had POAG and a factor of 4-15 if a sibling had POAG (Kanski 1999; Wolfs 1998). The lifetime risk of glaucoma was 22% in relatives of patients with glaucoma, almost 10 times higher than in controls (2.3%), yielding a risk ratio for glaucoma of 9.2 (95% CI 1.2-73.9) (Wolfs 1998). The authors suggest that at least 1/6 of all glaucoma in the general population may be caused by a genetic component, while other non-genetic factors determine overall occurrence to a great extent. Although the results were statistically significant, the number of cases was small (n=48), which reduced statistical power and created wide confidence intervals (Wolfs 1998).

A more recent study objectively diagnosed POAG by dividing patients into three categories using IOP, field and disc changes (Nguyen 2000). The investigators found that siblings of POAG patients had the highest risk (64.7%) of developing POAG compared with children (13.2%) or other blood relatives (22.2%). The authors attributed the higher proportion of affected family members in the study to possible inclusion of families with two or more members with POAG, thereby introducing selection bias in favour of heritable forms of the disease. Another important shortcoming of the study is the poor reliability of perimetry and the fact that only 37.2% of relatives returned for repeat visual field testing (Nguyen 2000). Nevertheless, the study supports the view that family history is a strong risk factor for developing POAG.

Additional evidence of a genetic origin is supported by the familial occurrence of several independent ocular parameters associated with POAG, such as increased IOP level, abnormal optic cup size and reduced facility of aqueous outflow (Tielsch 1991), which appear to be genetically determined (Armaly 1967; Armaly 1968). It is interesting to note that in the Rotterdam study, enlarged cup-disc ratio, not IOP, was the earliest and most prominent feature of familial aggregation (Wolfs 1998).

The accuracy of a reported family history of glaucoma was investigated by McNaught and colleagues (2000), who questioned 41 index cases about their prior knowledge of any family history of POAG and compared this to the rate of glaucoma identified as a direct result of examination. Even in large pedigrees, 27% of previously diagnosed POAG patients were unaware of their positive family history, suggesting that a higher percentage of adult POAG may be inherited than reported (McNaught 2000). In a greater Toronto multiethnic population, the accuracy was found to be highest for first-degree relatives (parents/siblings/children) and lower for second-degree relatives (grandparents/aunts/uncles) or third-degree relatives (great-grandparents, great-aunt/uncle, first cousins) (Williams-Lyn 2000).

Indeed, "a positive family history is a useful risk marker for developing POAG, but is subject to recall, selection and survival bias as well as community under-diagnosis of glaucoma which tend to underestimate the genetic influence on the prevalence of the disease" (Mitchell, in-press). Thus it appears that POAG is often inherited, probably in a multifactorial manner. The responsible gene is believed to show a lack of penetrance and a variation in expressivity in some families, and often exhibits features of local founder effects (Brezin 1998) in different populations (Pang 2000; Fingert 1999).

I.VI *GLAUCOMA GENETICS*

Recent advances in molecular genetics and genetic mapping have resulted in an expanding number of novel genes being identified (Lichter 1994; Della 1996).

In 1993, studies by Johnson and associates (1993) and Sheffield and associates (1993) of an American family with, primarily, JOAG led to mapping of the first POAG locus (Glaucoma 1A or GLC1A) to chromosomal region 1q21-q31 (**Figure I.5**). Linkage of JOAG to GLC1A was confirmed by later studies of additional families (Johnson 1996; Richards 1994; Graff 1995; Wiggs, 1995). Subsequent studies showed that GLC1A is also responsible for a subset of adult-onset POAG (Meyer, 1996; Morissette 1997).

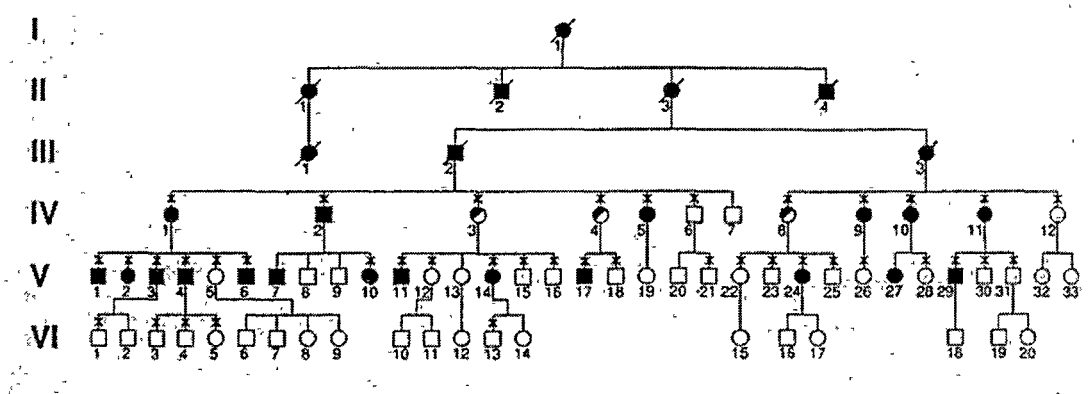


Figure I.5 A pedigree of autosomal dominant juvenile glaucoma. Individuals affected by glaucoma are indicated with filled symbols. Half-filled symbols represent ocular hypertension. X = individuals examined by the original authors (Johnson et al, 1993).

In 1997, Stone and 14 colleagues from seven laboratories reported the identification of a trabecular meshwork-induced glucocorticoid response (TIGR) gene associated with JOAG (**Figure I.6**). One of its proteins, originally called TIGR, showed time-dependent induction with dexamethasone treatment over several weeks (Polansky, 1997). In the same year, Kubota and associates (1997) discovered a protein associated with the cytoskeleton in the retina, which they termed myocilin (MYOC) because it shared homologous regions with myosin. This turned out to be the same gene and protein that Nguyen and colleagues (1998) had described and named TIGR. In 1998, the Human Genome Organization Genome Database Nomeclature Committee assigned the gene the name myocilin, abbreviated MYOC (Craig 1999).

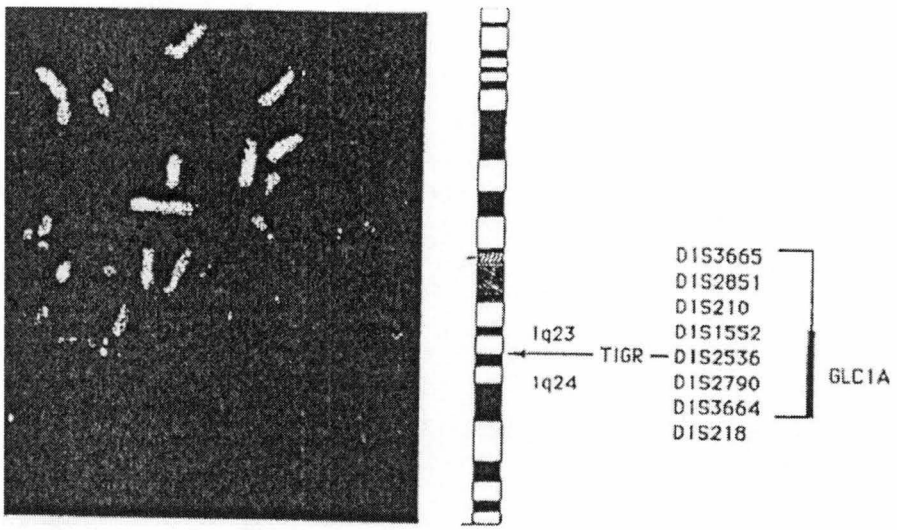


Figure I.6 TIGR gene (Johnson, 2000).

To date, over 26 mutations in MYOC (previously TIGR) have been described in some cases of adult-onset POAG (Johnson 2000). Indeed, other genetic mutations have also been reported in other forms of glaucoma as summarised in **Table I.2**. For example, primary congenital glaucoma is associated with mutations in the CYP1B1 gene on chromosome 2p21 (GLC3A) (Stoilova, 1997) and an unidentified gene on chromosome 1p36 (GLC3B)(Akarsu, 1996). Mutations in the FKHL7 gene on chromosome 6p25 is described in patients with Axenfeld-Rieger anomaly, which has at least 3 genetic loci (Craig 1999; Alward 2000).

Table 1.2 Summary of genetic inheritance of glaucoma (Craig 1999; Raymond 1997).

Glaucoma type	Locus	Location	Gene
Primary open-angle glaucoma			
JOAG & adult-onset POAG	GLC1A	1q24.3-q25.2	MYOC/TIGR
POAG (adult onset)	GLC1B	2cen-q13	NYI
POAG (adult onset)	GLC1C	3q21-q24	NYI
POAG (intermediate onset)	GLC1D	8q23	NYI
POAG (adult onset LTG)	GLC1E	10p15-p14	NYI
POAG	GLC1F	7p35-36	NYI
Primary congential glaucoma			
	GLC3A	2p21	CYP1B1
	GLC3B	1p36	NYI
Developmental glaucoma			
Rieger syndrome	RIEG1		
AD iris hypoplasia		4q25	PITX2
Iridogoniodysgenesis (IGD) syndrome	IRID2		
Axenfeld-Reiger anomaly			
Iris hypoplasia	RID1	6p25	FKHL7/
Familial glaucoma IGD			FREAC3
Familial glaucoma with GD			
Rieger syndrome	RIEG2	13q14	NYI
Other types			
Nail-patella syndrome	NPS1	9q34	LMX1B
Pseudoexfoliation syndrome		2p16	NYI
Pigment dispersion syndrome (PDS)	GPDS1	7q35-36	NYI
PDS	GPDS2	18q11-21	NYI

AD - autosomal dominant; **GD** - goniodysgenesis;; **JOAG** - juvenile-onset open-angle glaucoma; **LTG** - low-tension glaucoma; **NYI** - not yet identified; **POAG** - primary open-angle glaucoma.

I. VII *PRIMARY OPEN-ANGLE GLAUCOMA GENETICS*

Reports have confirmed that mutations in GLC1A are also responsible for approximately 3-5% of adult POAG in the general population (Morissette 1997; Meyer 1996; Alward 1998; Fingert 1999). In a recent collaborative study, the specific mutations found in different populations varied, mostly due to local founder effects; however, the overall frequency of disease-causing mutations in MYOC was similar across the five populations representing three racial groups (Fingert 1999).

The majority of mutations described have been missense mutations in the third exon, encoding an evolutionarily conserved olfactomedin-homology domain of GLC1A (Adam 1997). Specific mutations appear to correlate with severity of glaucoma and may act by a dominant negative mechanism. The most common mutation (Gln368STOP) was found to be associated with POAG with mean age of onset of 59 years and mean highest IOP of 30 mmHg (Alward 1998; Allingham, 1998). This mutation causes a relatively mild form of glaucoma compared to most other mutations in the GLC1A gene, such as the Thr377Met mutation (Alward 1998; Mackey, in-press) and the Thr448Pro mutation (Yokoyama 1999). It has already been shown that the Gln368STOP mutation has penetrance which increases with advancing age (Angius 2000; Craig 2001). However, further studies are needed to establish whether additional factors are required for the development of glaucoma.

In some pedigrees, the phenotype showed considerable variability in severity and age of onset, with some genotypically affected members exhibiting adult-onset POAG (Dubois 1997; Meyer 1996). Moreover, a study in a very large pedigree with 71 affected individuals showed that an Asp480Lys mutation segregates with POAG (Brezin, 1998). There is now a belief that other factors (genetic or environmental) may modify the phenotype of GLC1A-linked glaucoma. Indeed, genetic heterogeneity of POAG and linkage to GLC1A confer a highly increased risk of not only developing POAG but also having severe glaucomatous optic neuropathy (Brezin 1997).

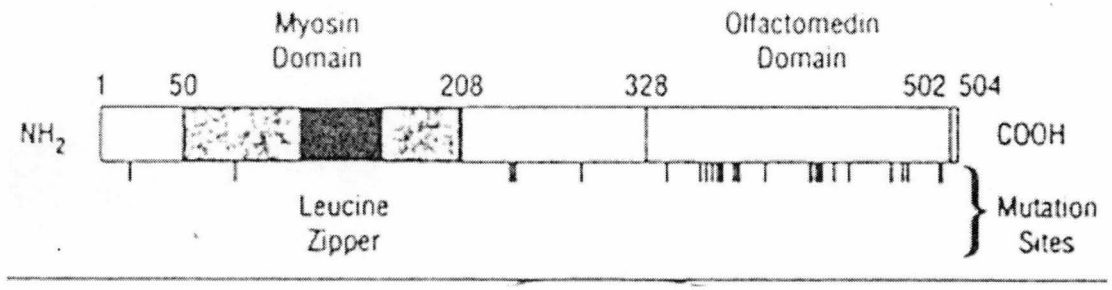


Figure I.7. Schematic diagram of human myocilin protein (Johnson, 2000).

The pathophysiology of myocilin is yet to be elucidated. The GLC1A gene (**Figure I.7**) consists of three exons that encode a 504 amino acid protein with an olfactomedin-like domain and a leucine zipper motif hypothesised to mediate protein-protein interactions

(Shimizu 2000). Myocilin is an endogenous glycoprotein with molecular weights of 66 kD (glycosylated form) and 55 kD (nonglycosylated form)(Polansky, 1997) that may function as a heat shock protein which protects tissues during stress. It is normally present in a variety of ocular and nonocular tissues, including trabecular meshwork, cornea, retina, optic nerve, ciliary nerves, and the heart, skeletal muscle, stomach, thyroid and lung (Fingert 1998). Expression studies have shown that myocilin is shown in the trabecular meshwork of both normal and glaucomatous eyes (Takahashi et al 2000), where the intracellular protein colocalises with microtubules (Mertts et al 1999). However, it may be present in more regions and have more intense labelling in the glaucomatous eyes (Lutjen 1998).

Recent studies suggested that GLC1A mutations led to elevated IOP by trabecular dysfunction and reduced aqueous outflow (Nguyen 1998; Lutjen 1998; Wilkinson 1998). Although myocilin is also expressed in the ciliary body, which is responsible for aqueous production (Craig 1999). A variant in the promoter region of the nitric oxide synthase (NOS) gene has been found in a significant percentage of familial POAG. Although this genetic polymorphism may not be of physiologic significance, NOS catalyses endothelium-derived nitric oxide, a potent modulator of vascular tone in ophthalmic artery, which can contribute to ischaemic damage of optic nerve head (Adam 1997).

There have been significant impediments to establishing linkage for autosomal dominant POAG in some pedigrees (Coote 1966) for the following reasons:

- 50%-60% of glaucoma is not diagnosed in the community (Wensor 1998; Weih 2001)
- Diagnostic uncertainty of glaucoma and subtle phenotypes
- Late onset and late age of diagnosis
- Affected parents are often deceased
- Little contact with affected cousins or second cousins (Mackey, personal communication 2001).

Despite these problems, **Table I.2** illustrates that significant linkage has been established to at least five chromosomal regions in addition to GLC1A in some families with POAG, including markers in the region 2cen-q13 (designated GLC1B)(Stoilova 1996; Allingham 1998), 3q21-24 (GLC1C)(Wirtz 1997), 8q23 (GLC1D)(Trifan 1998), 10p15-p14 (GLC1E)(Sarfarazi 1998) and most recently 7q35-36 (GLC1F)(Wirtz 1998). The contribution of these loci is yet to be determined as the specific genes have not yet been identified but they tend to support an autosomal dominant mode of inheritance. It is interesting to note that some cases of GLC1B and GLC1E are associated with normal-tension glaucoma, suggesting a different pathophysiological mechanism (Craig 1999).

I.VIII *GLAUCOMA INHERITANCE IN TASMANIA*

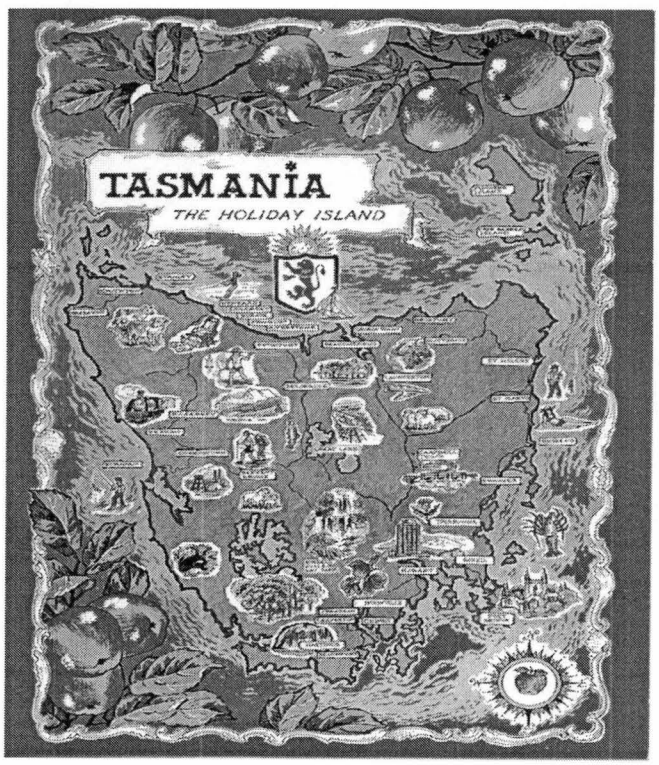


Figure I.8. Tasmanian map

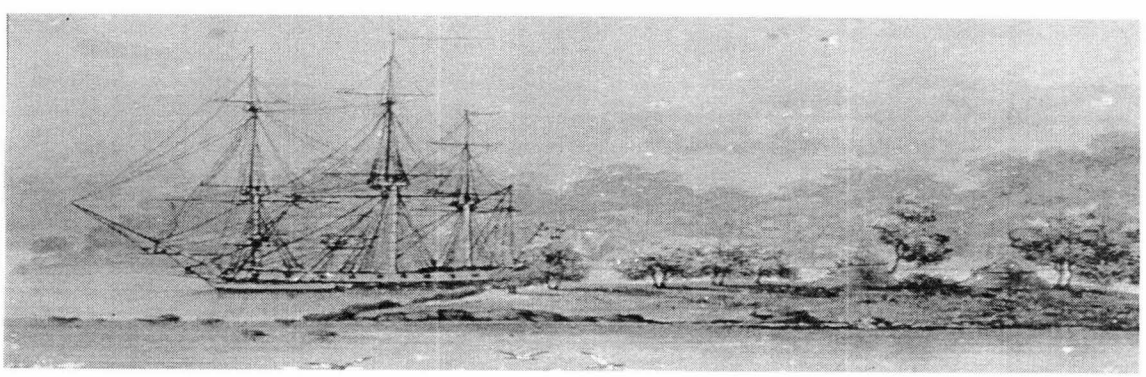


Figure I.9. *Lady Nelson* was one of the earliest tall ships transporting convicts from United Kingdom to Tasmania

An island community such as Tasmania (**Figure I.8**) or Iceland is highly suitable for genetic research. Finding large pedigrees was made easier by the following features (Mackey, personal communication 2001):

1) Tasmania is a somewhat isolated Australian island state that was settled by Europeans in 1803. Today, it has a 'captive' population of approximately 470,000 (**Figure I.9**).

2) A large Founder effect from early settlers with large families within the population (**Figure I.10**) (Brezin 1998).

3) Comprehensive genealogical records, with 1 in 30 Australians able to trace their family tree back to the original immigrants. Active genealogical societies have created a computerised database of births, deaths and marriage records.

4) A high standard of ophthalmic care to over 5000 Tasmanians affected by glaucoma, allowing a reliable diagnosis of glaucoma and ensuring at least 50% of cases are identified.

The Glaucoma Inheritance Study in Tasmania (**GIST**) score was developed to give a relative likelihood of glaucoma to family members as it reflects objective physician grading of intraocular pressure, optic disc changes and visual field defects (Coote 1996). Please refer to chapter IV for more details.

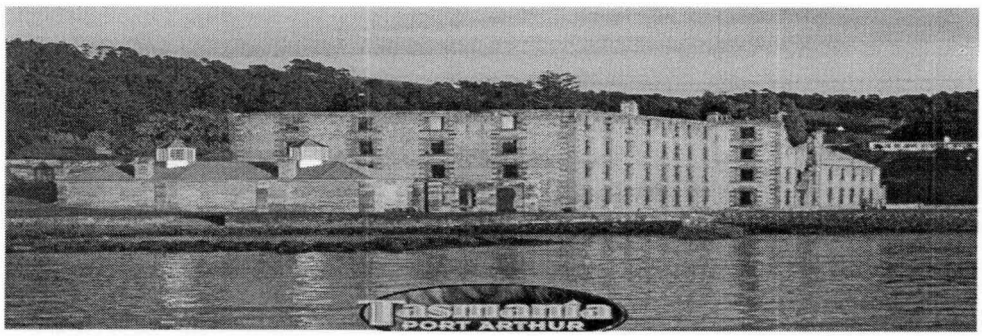


Figure I.10. Port Arthur – early convicts settlement in Tasmania.

I.IX *SUMMARY*

- Glaucoma is a highly heterogeneous group of eye disorders, the most common type of which is POAG in developed countries.

- Known as the "sneak thief of sight", up to 50%-60% of people with glaucoma in the community remain undiagnosed (Mitchell 1996; Quingley 1996; Wensor 1998).

- Over 50% of all glaucoma is familial (Mitchell 1996; McNaught 2000).

- A positive family history of glaucoma gives a 3-fold increase in risk of developing POAG (Leske 1996; Tielsch 1994; Hourihan 1999).

- Over 27% of diagnosed POAG patients are unaware of their positive family history (McNaught 2000).

- Mutations in GLC1A (MYOC) are identified in approximate 3-5% of adult POAG in the general population, which may exhibit local founder effects (Fingert 1999).

- Significant linkage has also been established to at least five additional loci (GLC1B-F).

- The pathophysiology of myocilin is unclear, but the current multifactorial model suggests that other factors (genetic and environmental), as yet uncharacterised, may modify the expression of the POAG phenotype in GLC1A pedigrees.

In chapter II, the Review of Related Literature highlights the current understanding of the associations between total POAG and various clinical risk factors; in chapter III, the Conceptual Framework and Hypotheses are stated; and in chapter IV, the Methodology is described.

In chapter V, the Results and Statistical Analysis comparing familial and sporadic glaucoma groups are presented; in chapters VI – XV, the Results, Statistical Analysis & Discussion on various clinical risk factors are dissertated; and in chapter XVI, the Conclusion, Implications and Recommendations for future research and practices are examined.

CHAPTER II

REVIEW OF RELATED LITERATURE

"That men do not learn very much from the lessons of history is the most important of all the lessons that history has to teach."

(Aldous Huxley)

II.I *OCULAR RISK FACTORS*

II.I.I *Intraocular Pressure (IOP)*

Elevated IOP is generally believed to be a major risk factor for glaucoma (Vaughan 1992; Berson 1993; Leske 1995; Tielsch 1991; Leske 1983). The risk of optic nerve head damage increases as the IOP increases. For IOP of 16-21 mmHg, the prevalence of POAG is 1.5%. As IOP rises to 22-29 mmHg, the prevalence is 8%. At IOPs of over 30 mmHg, the prevalence rises to 25% (Kanski 1999). As found in the Blue Mountains Eye Study (Mitchell 1996), the steady increase in POAG prevalence with increasing IOP and the steep rise in risk with IOP above 23 mmHg suggests a causal relation, particularly at high IOPs. A similar trend was reported in the Baltimore Eye Survey (Tielsch 1991), which confirms a continuous nature of the IOP-glaucoma relationship, with no clear IOP level to distinguish between so-called high- and low-tension glaucoma (Mitchell 1996). POAG may occur throughout the range of IOP levels (Leske 1983; Klein 1991; Sommer 1991). Conversely, glaucomatous changes do not always occur with raised IOP (ocular hypertensive individuals), and glaucomatous change can be observed in eyes with IOP within the normal range (normal-tension glaucoma) (Berson 1993).

A possible limitation of these studies is the "snapshot nature of a single presenting IOP measure, which may not represent the peak or mean IOP or provide information on the duration of any elevation." This may explain the relatively low proportion of glaucoma cases with elevated IOP in the Blue Mountains Eye Study (Mitchell 1996). Furthermore, the lack of a one-to-one relationship between elevated IOP and glaucomatous change (Dielemens 1995), the occurrence of increased IOP after initial observation of disc abnormalities, and the lack of a clear unequivocal demonstration of the efficacy of IOP lowering in preventing progressive damage simply suggest other factors contribute to the development of glaucoma.

The Barbados Eye Study (Wu 1997) found that high systolic blood pressure (hypertension), diabetes, and older age were positively associated with IOP ($P<0.01$), as were female gender, darker complexion, higher pulse rate, higher body mass, seasonality, family history of glaucoma, current alcohol use, and current smoking. As a high IOP is a major risk factor for POAG, it would be reasonable to speculate that similar associations occur for both conditions. However, studies to date have demonstrated that only advancing age and self-reported family history of glaucoma are significantly associated with both ocular hypertension and POAG, suggesting that gene-environment interaction is important in the pathogenesis of glaucoma (Wu 1997). Indeed, 7-8% of the population over the age of 40 years have IOPs greater than 21 mmHg, but only 1% of individuals with ocular hypertension (OH) will develop glaucomatous visual field loss each year (Kanski 1999). More recently, the Blue Mountains Eye Study reported that after adjusting for systolic blood pressure and confounding effects of diabetes, glaucoma family history and myopia, age was not significantly associated with IOP ($p<0.29$) (Rochtchina 2002).

II.II *NON-OCULAR RISK FACTORS*

II.II.I *Age*

Earlier studies demonstrated a higher prevalence of POAG with advancing age (Hollows 1966; Kahn 1980; Mason 1989), and these have been confirmed by many recent population-based cross-sectional studies (Leske 1995; Wang 1997; Klein 1992; Dielemens 1994; Mitchell 1996; Wensor 1998; Angius 2000; Klaver 1998; Mukesh 2002). The Collaborative Glaucoma Study (Armaly 1980) also identified age as the major predictor of glaucoma incidence.

Although an independent age effect on IOP is controversial (Wu 1997; Rochtchina 2002), it is likely that the optic nerves of the elderly are more susceptible to damage for reasons other than higher pressure (Wilson 1994).

II.II.II *Gender*

A few incidence studies noted higher rates of POAG in women (Bengtsson 1989; Teikari 1989), but population-based studies have yielded conflicting results on sex-specific glaucoma prevalence. There is a higher rate in white men in some studies (two-fold male to female risk in the Framingham Eye Study (Leibowitz 1980) and the Long Island study reported a male odds ratio of 1.69 (Leske 1996)), but not in others (Mason 1989; Tielsch 1991; Leske 1983; Kahn 1980; Hollows 1966; Klein 1991; Alward 2000).

Furthermore, the Blue Mountains Eye Study found a higher prevalence of POAG in Australian women in comparison to men (OR 1.5; CI 1-2.2)(Mitchell 1996). In contrast, the Melbourne Visual Impairment Project found no relationship between POAG and gender (Wensor 1998; Weih 2001).

The Barbados Eye Study found a higher rate of POAG in black men, who show age-specific differences between gender. However, the authors concluded that this could be due to differences in reporting secondary to some unidentified bias such as differences in reporting family history, or there may exist a real gender difference in risk (Leske 1995). Further corroborating evidence is needed.

II.II.III *Race*

Clinic-based studies have reported a higher proportion of black patients in glaucoma clinics in comparison to general eye clinics (Martin 1985; Wilson 1987), and a greater proportion of black patients with glaucoma undergoing glaucoma surgery as compared to medical therapy (Coulehan 1980).

Many large population-based studies examined prevalence of POAG in populations that are largely white communities, including the United Kingdom (Hollows 1966; Coffey 1993), United States of America (Kahn 1980; Kahn 1980; Klein 1992), Sweden (Bengtsson 1981), Holland (Dielemans 1994) and Australia (Mitchell 1996; Wensor 1998).

A number of recent surveys have also included Asian (Shiose 1991), multiracial (Tielsch 1991) or black (Leske 1994) communities in which at least a 4-5 times higher prevalence rate has been found (Flammer 1998).

II.II.IV *Demographics*

The Melbourne Visual Impairment Project reported no significant association between age, gender, or residential area and use of glaucoma medications (Weih 1998). Moreover, a previous diagnosis of open-angle glaucoma (OAG) or OH was not significantly associated with decreased socio-economic status, the use of community support services or impaired mobility (Hourihan 1999).

II.III *CLINICAL RISK FACTORS OF POAG*

Several epidemiological studies have began to identify groups at risk of developing POAG, and have led to formulation of hypotheses and the elucidation of potential risk factors. Although no aetiological factor is yet identified, some risk factors such as hypertension and diabetes mellitus may be modifiable through intervention and disease-prevention strategies. Others, such as family history, age, gender and race, cannot be modified, but do facilitate identification of those at highest risk of glaucoma blindness for whom close medical supervision and follow-up is beneficial. This may also affect rate of progression of disease and be helpful in guiding therapy (Wilson 1994).

II.III.I *Systemic Blood Pressure (Hypertension)*

The data regarding an association between POAG and hypertension are conflicting. Many studies have reported a positive association between increased blood pressure and raised IOP (Leske 1983; Bulpitt 1975; Kahn 1977; Klein 1981). However, there is no consensus either regarding the possible relationship of POAG to high blood pressure or on the possible effects of antihypertensive treatment (Wilson 1987; Drance 1973; Morgan 1975). Furthermore, it has been criticised that earlier clinic-based studies reporting positive association may result from selection biases or from the confounding effect of age, IOP or other variables.

Results from population-based studies have also differed. In the Baltimore Eye Survey, there was no significant association between hypertension and POAG. Race-adjusted odds ratios tended to be lower at younger ages and higher at older ages, suggesting that hypertension is protective at ages under 60 and damaging at older ages (Tielsch 1995). However, the results were not statistically significant.

In the smaller Rotterdam POAG Study (n=42), a positive relationship between hypertension and POAG was found only in cases with high IOP; however the odds ratio was not significant (Dielemans 1995). Furthermore, the Barbados Eye Study (n=302 definite cases of POAG) found that an elevated systolic blood pressure, regardless of treatment, was associated with POAG at ages under 70 years; however the association was weak and did not persist after age-sex adjustment or multivariate analysis (Leske 1995). More recently, the

Long Island Study also did not support the hypothesis that systemic hypertension independently increases the risk of POAG field defect (Leske 1996).

It is of interest to note that both the Barbados Eye Study and the Long Island Study found a significant association between POAG and low perfusion pressure (low diastolic blood pressure-IOP differences). An association between low systolic and diastolic blood pressure to IOP ratios has also been documented (Leske 1995; Leske 1996). The role of vascular risk factors is consistent with findings of low blood pressure to IOP ratios, but this could be explained by the high IOP in POAG rather than by a relatively low blood pressure. Moreover, the relationship between systemic blood pressure and IOP may not accurately reflect the local perfusion pressure at the optic disc. At present, therefore, there is insufficient evidence to support the role of hypertension as a strong independent risk factor for POAG.

II.III.II *Diabetes Mellitus*

The association between diabetes mellitus and POAG is still controversial.

A number of studies have reported an increased prevalence of individuals with abnormal glucose metabolism among patients with glaucoma compared to the general population (Armstong 1960; Armaly 1969), and subsets of individuals with abnormal glucose metabolism have significantly greater risk of developing glaucoma (Armaly 1969; Katz 1988).

Furthermore, an elevated mean IOP in patients with diabetes compared to those without diabetes has also been documented (Becker 1971; Kass 1978; Klein 1994). However, a similar number of studies did not support such associations (Bengtsson 1981; Armaly 1980; Mapstone 1985; Bouzas 1971). Tielsch and associates (1995) have suggested that the positive association found in some previous clinic-based studies was due to selection bias. As both diabetes and glaucoma are commonly underdiagnosed, there is significant potential for selection bias when ascertainment is based only on self-presentation to a doctor. People with diabetes are more likely to be seen by an ophthalmologist than people without diabetes and hence are more likely to have glaucoma detected.

To date, only a few population-based studies have examined the relationship between the two disorders (Kahn 1980; Kahn 1980; Klein 1994; Tielsch 1995; Dielemens 1996; Mitchell 1997). The Baltimore Eye Survey failed to confirm a significant association between diabetes and POAG in 161 black and white participants with definite or probable POAG (age-race adjusted OR 1.01) (Tielsch 1995). After stratifying participants into previous and newly-diagnosed glaucoma cases, the study found a weak association between diabetes and POAG for patients who knew they had glaucoma (age-race adjusted OR 1.71), but no association for those with newly diagnosed glaucoma (age-adjusted OR 0.60). Thus giving support to the likelihood of selection and recall bias in earlier studies.

In contrast, the Rotterdam Eye Study found a threefold increased presence of high-tension glaucoma (OR 3.11; CI 1.2-8.66) among newly diagnosed diabetic patients (both men and women) (Dielemens 1996). The Blue Mountains Eye Study which had 108 glaucoma cases (Mitchell 1997), diagnosis of glaucoma was made if matching visual field and optic disc cupping were present, independent of any prior glaucoma diagnosis or therapy or any particular IOP level. This minimised diagnosis bias. Fasting plasma glucose levels were ascertained, and if higher than 7.8 mmol/l, diabetes was diagnosed. Similar to the results reported in the Beaver Dam Study (Klein 1994), glaucoma prevalence was approximately doubled in people (both men and women) with diabetes diagnosed from history or biochemical evidence of elevated plasma glucose levels (5.5%), compared with those without diabetes (2.8%; age-gender adjusted OR 2.12; 95%CI 1.18-3.79). After adjusting for IOP (higher of the two eyes), age and gender, the association between glaucoma and diabetes persisted, albeit slightly reduced (OR 1.77; CI 0.96-3.26).

Conversely, in the same study, diabetes was present in 13% of people with glaucoma (and 16.7% of those with a previous diagnosis of glaucoma; OR 2.82 CI 1.35-5.87) compared with 6.9% of those without glaucoma. A weaker, but non-significant association between newly diagnosed glaucoma and diabetes remained (OR 1.47; CI 0.58-3.73). This seems to support the suggestion that selection or discovery bias, such as more frequent eye examinations amongst diabetic patients, may partly explain the association between diabetes and glaucoma. However, 67% of those with both diseases had glaucoma diagnosed before the diabetes, suggesting that selection bias is probably less important than anticipated (Mitchell 1997).

The role of genetic factors in the association has been questioned by the study. The significant relation between diabetes and glaucoma persisted after adjusting for family history of diabetes, age and gender in a logistic regression (OR 2.41; CI 1.30-4.44). The relation was also maintained after adjusting for a family history of glaucoma, age and gender (OR 2.22; CI 1.24-4.53) (Mitchell 1997).

II.III.III *Migraine Headache*

In a neurologic study of 27 patients, Corbett and associates (1985) noted that nearly half of their patients with low-tension glaucoma gave a history of common or classic migraine headache. In their subsequent case-control study (Phelps 1985), the authors found that the increased prevalence of headache in the low-pressure glaucoma group compared with the normal group was statistically significant for individuals aged 70 years or older (P=0.04).

Several researchers have hypothesised that the glaucomatous damage of optic nerve head may be caused by migraine-related transient vasospastic alterations of cerebral or meningeal blood flow, ischaemic vascular diseases (Drance 1973) and blood coagulopathies (Klaver 1985; Winder 1977). However, in a case-control study, Usui (1991) failed to find a statistically significant correlation in migraine prevalence between either low-pressure glaucoma, POAG, or normal subjects. Moreover, a multivariate study found no difference between low-tension and high-tension glaucoma groups with respect to organic vascular pathologic findings (Carter 1990). In the population-based Beaver Dam Eye Study, no

association between POAG and migraine headache was found (Klein 993). Despite continuing controversy, it is reasonable to suggest that local perfusion pressure at the nerve head is an important variable in the development of POAG, regardless of IOP (Wilson 1994).

In a more recent Blue Mountains Eye study, for all age groups combined, there was no significant association between typical migraine headache (16.7% reported history of migraine) and POAG (16.6%) (OR 1.3; 95% CI 0.8-2.2), after multivariate adjustment including glaucoma family history, diabetes, pseudoexfoliation, and hypertension (Wang 1997).

After stratifying the subjects into 10-year age groups, Wang and associates found increased odds for POAG among people having a history of typical migraine and aged 70-79 years (OR 2.5; CI 1.2-5.2), after adjusting for variables associated with POAG (Wang 1997). Furthermore, the association was slightly stronger for high-pressure POAG (>21mmHg) cases (OR 2.7; 1.1-5.6) (Wang 1997).

The authors concluded that other influences such as genetic factors might be important and if an association between migraine and POAG exists, it is likely to be only modest (Wang 1997).

II.III.IV *Cigarette Smoking*

Glaucoma has been reported to be associated with increased alcohol consumption (Kahn 1980) and cigarette smoking (Wilson 1987). However, in the Blue Mountains Eye study, smoking was demonstrated not to be significantly associated with POAG, and adding smoking (current versus non-current or ever versus never) into the multivariate model comparing migraine and POAG did not alter results (Wang 1997).

II.III.V *Corticosteroid Responsiveness*

Many studies have demonstrated that the use of oral or topical ophthalmic corticosteroids in a minority of people, called 'steroid responders', can lead to varying degrees of elevated IOP and predispose them to developing POAG (Becker 1963; 1965; Williamson 1969; Mitchell 1999). The normal population can be classified into three groups according to their IOP responsiveness to daily topical administration of a potent corticosteroid (betamethasone or dexamethasone) for 4-6 weeks (Clark 1995; Richardson 1997). The majority (two-thirds) of people in the population show no response at all (Armaly 1965; Becker 1964; Foon 1977). These "non-responders" develop pressure rises of less than 6 mmHg and IOPs of less than 20 mmHg. On the other hand, 4-6% of the population are categorised as "high responders", demonstrating IOP increases of more than 15 mmHg and IOPs greater than 31 mmHg. The intermediate group of "moderate responders", comprising approximately one-third of the population, exhibit more delayed and lower pressure rises of 6-15 mmHg with IOPs between 20 and 31 mmHg.

An earlier study reported that nearly all persons with a diagnosis of POAG were moderate-to-high responders to corticosteroids (Becker 1964). Ninety percent of patients with POAG were high responders to a 6-week course of topical ocular betamethasone, during which their IOPs showed marked elevation (>30mmHg). The remaining 10% of patients with POAG showed moderate response with a moderate elevation of IOP (22-30mmHg). Steroid responders in the normal population were also shown to have a higher risk of developing POAG over time (Lewis 1988; Kitazawa 1981).

A genetic basis for steroid-induced ocular hypertension was postulated by some investigators in the 1960s (Becker 1964; Armaly 1967), but disputed by others (Francois 1966). Clinic studies have demonstrated that inheritance of a steroid response may have an autosomal recessive pattern and may be associated with inheritance of glaucoma (Davies 1968; Bartlett 1993) in a rather complex manner (Clark 1995). It is interesting that both siblings and offspring of patients with POAG have increased responsiveness to steroids (30 and 25% respectively are high responders) (Richardson 1997). Recently, the discovery of the trabecular meshwork-induced glucocorticoid response (TIGR) gene or MYOC at the GLC1A locus and its multiple disease-causing mutations in some 5% of hereditary glaucoma has added further support for the genetic basis (Sheffield 1993; Stone 1997; Fingert 1999).

Users of inhaled corticosteroids may also be at increased risk of OH and glaucoma. This association was noted in a health insurance database study in Quebec (Garbe 1997; Garbe 1998). The Blue Mountains Eye Study reported a strong association between ever use of inhaled corticosteroids and findings of glaucoma or OH in persons with a family history of glaucoma (OR 2.6; CI 1.2-5.8). Moreover, the risk increased with higher doses (OR 6.3; CI 1.0-38.6) for persons who used more than four puffs per day (Mitchell 1999). However, no significant association was found for persons with no family history of glaucoma, adding further support for a genetic mechanism in the association.

II.IV *SUMMARY*

- POAG is positively associated with age and IOP. Black communities and female gender are probable risk factors of POAG.

- Conflicting evidence exists on the association between POAG and systemic hypertension. There is, however, more supportive evidence of an association between POAG and low perfusion pressure (low diastolic blood pressure – IOP difference), and between POAG and low blood pressure to IOP ratios (Leske 1986; Leske 1995). These two latter relationships are not examined in the present study.

- The current literature suggests that POAG and diabetes mellitus are positively associated. There is a two- to three-fold increased risk of POAG among diabetic patients and a two-fold increase in risk of diabetes among POAG patients (Dielemens 1996; Mitchell 1997).

- The association between POAG and migraine is less consistent and, if it does exist, it is likely to be modest (Wang 1997). Ocular vasospasm is a likely risk factor for POAG (Flammer 1992; Drance 1988), and may be associated with generalised vasospastic tendency manifested in migraine, Raynaud's phenomenon (cold extremities) and Prinzmetal angina (atherosclerosis).

- There is no strong evidence for an association between POAG and smoking (Wang 1997).

- A preponderance of studies has demonstrated that nearly all glaucoma patients are moderate-to-high responders to topical corticosteroids. Steroid responders in the normal population are also at an increased risk of developing POAG (Lewis 1988; Kitazawa 1981). Use of inhaled corticosteroids is also associated with POAG or OH (Mitchell 1999).

- Despite the number of large-scale cross-sectional epidemiologic studies examining the relations between total (familial and sporadic) glaucoma and various clinical risk factors, no study has yet investigated the potential links between familial POAG and the above clinical risk factors. This study aims to examine all 'glaucoma' patients in Tasmania and categorises them into familial and sporadic subgroups based on genealogic data and objective examinations. The prevalence of various risk factors in the two groups is compared and potential modifiers in the familial group are analysed.

CHAPTER III

CONCEPTUAL FRAMEWORK & HYPOTHESIS

"It is a good morning exercise for a research scientist to discard a pet hypothesis everyday before breakfast. It keeps him young."

(Konrad Lorenz)

Strategies to eliminate the blindness toll of glaucoma must be aimed at identifying undiagnosed glaucoma cases in the community to achieve early diagnosis and management before the onset of vision loss. Given the significant risks associated with a positive family history of glaucoma, it is the primary aim of this thesis to:

* Seek evidence of differences in prevalence of known or potential risk factors between two populations classified as having glaucoma based on GIST scores of 0.7 or greater – the familial population defined by genealogic connection to known glaucoma sufferers and the sporadic population defined by the absence of genealogic connection to known glaucoma patients.

Adjustment will be made for possible gender and age differences and for potential interactions amongst the various risk factors.

If there is evidence against the null hypothesis of no difference in expected rates of risk factors between the two glaucoma populations, pairwise comparisons are employed to determine where the difference lie.

Further aims include:

1. to compare the prevalence of risk factors between the two glaucoma populations at GIST scores of 0.7, 0.8, 0.9 and 1.0;
2. to examine potential differences in the prevalence of risk factors between the sporadic glaucoma population and the familial glaucoma population at 1st, 2nd, 3rd and 4th degrees of connection (relationship) to family members with POAG;
3. to compare the prevalence of risk factors between the sporadic glaucoma population and the individual pedigrees of the familial glaucoma population; and
4. to establish if the prevalence of risk factors differ between a no glaucoma ('controls') population as defined by GIST scores of 0.5 or less and a total glaucoma (familial & sporadic) population as defined by GIST scores of 0.7 or greater.

CHAPTER IV

MATERIALS & METHODS

"You know my methods, Watson ..."

(Memoirs of Sherlock Holmes, Sir Arthur Conan Doyle)

This is a cross-sectional, retrospective study comparing the prevalence of various risk factors, such as hypertension, diabetes and migraines, in patients with familial POAG to the prevalence of the same risk factors in patients with sporadic or non-familial POAG, using a multi-stepped regression analysis.

IV.I *Study Population*

With the cooperation of ophthalmologists in Tasmania, an extensive review of the clinical notes of all patients attending all ophthalmic practices in the state was performed, thereby creating a glaucoma registry for each ophthalmologist. Surveys inviting glaucoma patients (index cases) to participate in the study were directly mailed to 3800 Tasmanian patients who had been investigated or treated for 'glaucoma' over the previous 15 years. Furthermore, in order to cover the entire glaucoma population, surveys were distributed throughout the state through ophthalmologists, optometrists, general practitioners, glaucoma support groups and pharmacies with additional general community awareness through local newspapers, radio and television media publicity.

Written informed consent (**Appendix A**) was obtained from patients to participate in the Glaucoma Inheritance Study, which was approved by the relevant ethics committees of the following institutions: The University of Tasmania (Hobart), the Royal Hobart Hospital (Hobart, Tasmania), and the Royal Victorian Eye and Ear Hospital (Melbourne, Victoria). This study is conducted in accordance with the Declaration of Helsinki and subsequent revisions.

Care was taken to ensure participants understood that they were under no obligations and were free to discontinue at any time. They were asked several times during the testing procedures if they were happy to continue.

In the surveys (**Appendix B**), participants provided information regarding their families and were asked if they had relatives with glaucoma and who their parents were. The index cases were then followed-up with questions about their grandparents and if any family tree had been traced in the family. With the assistance of a professional research genealogist using computerised family tree databases available in Tasmania, 309 pedigrees were reconstructed and the genealogy was extended by connecting with other index cases to complete family trees and to locate all descendants over 10 years of age to be invited for examination.

As reported earlier, there are overlaps of different glaucoma pedigrees through intermarriages (Sack 1996). The multiple genotypes in individuals who had family members affected with glaucoma on both the maternal and paternal sides of the family may partly explain the phenotypic heterogeneity in some families. Therefore, alternate parents or their families were also examined, particularly if the phenotype was atypical for the rest of the family.

The **exclusion criteria** were as follows:

- presence of glaucoma other than POAG, such as angle-closure or pigmentary glaucoma (n=12; 0.3%)
- <10 years of age, such as congenital glaucoma (n=223; 5.9%)
- associated syndromes of anterior segment dysgenesis, such as nail-patella syndrome and aniridia (n=26; 0.7%)
- presence of field defect or optic disc pathology other than POAG, such as macular degeneration, vascular/thrombotic events, optic disc drusen and cerebrovascular accident (n=26; 0.7%)
- glaucoma highly distinct from the pedigree phenotype (n=5; 0.1%)
- inability to complete study protocol including refusals and deaths (n=68; 1.8%)

3290 subjects (86.6%) participated in the 5-year study ending November 1998 (age range 10-106 years). 360 subjects were excluded, giving a final number of eligible participants of 2930 (77.1%).

These individuals were categorised into four groups based on the GIST score as follows:

Table IV.1 – Distribution of subjects into familial glaucoma, sporadic glaucoma, borderline and unaffected 'controls' groups based on GIST scores and genealogic data.

Unaffected GIST≤0.5	**Borderline** GIST=0.6	**Familial Glaucoma** GIST≥0.7	**Sporadic Glaucoma** GIST≥0.7	**Total**
742	486	854	848	2940

There are 1667 males (average age 65 years) and 1263 females (average age 66 years).

IV.II *Procedures/Instrumentation*

As the identity of all glaucoma genes and associated disease-causing mutations is not yet completed, we are still dependent on clinical examination for detection of glaucoma using the three standard tests of tonometry, visual field examination and optic disc analysis as previously validated (Vernon 1998).

Through the assistance of volunteer ophthalmologists, research fellows, orthoptists, nurses and medical students, large numbers of affected and unaffected members of extended families were examined. There were five masked examiners, each of whom assessed one parameter of glaucoma by following the standard clinical examination protocol for each patient (**Appendix C**); one member of the research team took a history, obtained consent, refracted and measured visual acuities; another examined visual fields; another measured IOP and performed gonioscopy; and two independent observers scored the optic discs, and finally fundus photographs and DNA via venepuncture were taken.

Participants attended various eye clinics throughout Tasmania or were visited at their homes if unable to attend a clinic. A detailed questionnaire (**Appendix D**) and standard interview were administered covering knowledge of family history, demographic data, medications (including drug names and frequency of use), and medical history of systemic disorders such as hypertension, diabetes, migraine, corticosteroid use and systemic vascular disease. Problems with vision, past eye disease or eye treatment, and ocular symptoms were also included. For accuracy, patients were asked to bring all their medications or their physicians' medical summaries to the interview.

The detailed bilateral eye examination included subjective refraction and best-corrected visual acuity using the Snellen chart **(Figure IV.1)**.

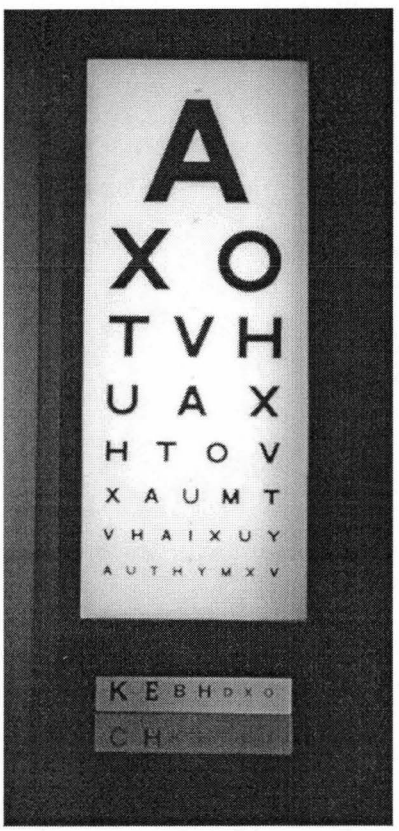


Figure IV.1. Snellen chart.

Seated IOP was taken using the standard calibrated Goldmann applanation tonometer (Haag Streit AG, Bern, Switzerland) with a drop of fluorescein 2.0% tear film (Chauvin Pharmaceuticals Ltd, Essex, UK) enhancement and local anesthetic on each eye **(FigureIV.2)**. The IOP was not standardised for time of day but the highest IOP record was used whenever possible to reduce the influence of diurnal variations. The IOP reading

was repeated if deemed unreliable. Attention was given to ensure the absence of tight clothing, particularly around the collar, as this could have potentially affected the venous pressure and influence IOP. This was followed by anterior segment examination with AC width assessed using the van Herick method (1969); any anterior chamber, iris, and lens abnormalities were recorded **(FigureIV.3)**. IOP from portable devices such as Perkins (Clement Clarke, Harlow Essex, UK) or Tonopen (Mentor, Norwell, MA, USA) were regarded as satisfactory if they were the only practical alternative (such as with the bed-bound or in extreme geographical isolation).

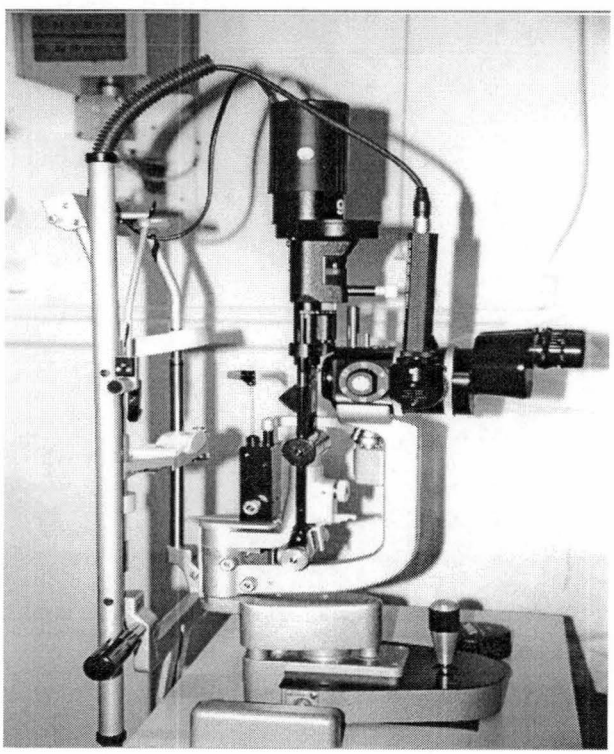


Figure IV.2. Slit lamp and Goldmann applanation tonometer

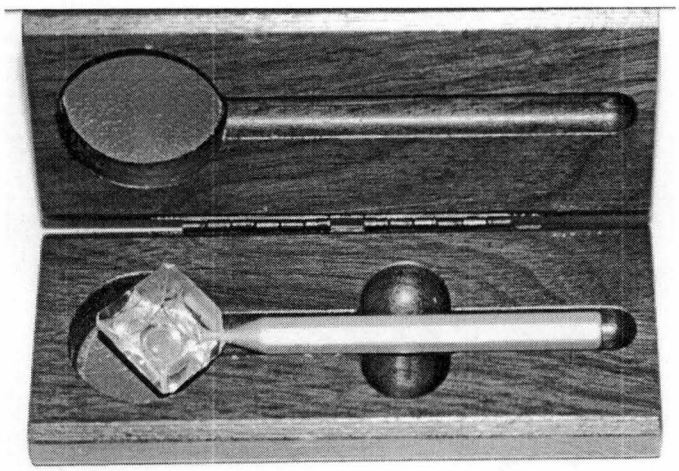


Figure IV. 3. Gonioprism for anterior chamber examination

Optic disc analysis **(FigureIV.4)** was performed with prior pupillary dilatation with tropicamide 1.0% and phenylephrine 10% (Chauvin Pharmaceuticals Ltd, Essex, UK) using both direct and indirect ophthalmoscopy, and a slit-lamp biomicroscope under magnifying binocular stereovision using a 78 or 90 dioptre noncontact lens or fundus contact lens. The following features were noted by two independent clinicians and were ranked according to the GIST scoring system (**Appendix E**):

- Size of scleral canal (horizontal and vertical)
- Presence and amount of peripapillary changes to retinal pigment epithelium and choroidal vasculature
- Consistency and depth of retinal nerve fibre layer up to one disc distance from the disc edge

- Vascular branching pattern
- Presence of 'Drance' type nerve fibre layer haemorrhages
- Neuroretinal rim width, consistency and colour
- Focal defects in the rim or pits not contiguous with the central cup
- The vertical and horizontal cup-disc ratio as judged on contour (noting the phenomenon of 'overpass cupping'), 'bayonetting' of emerging nerve head vasculature, widening of the interstices of the lamina cribrosa, and posterior bowing of the lamina.

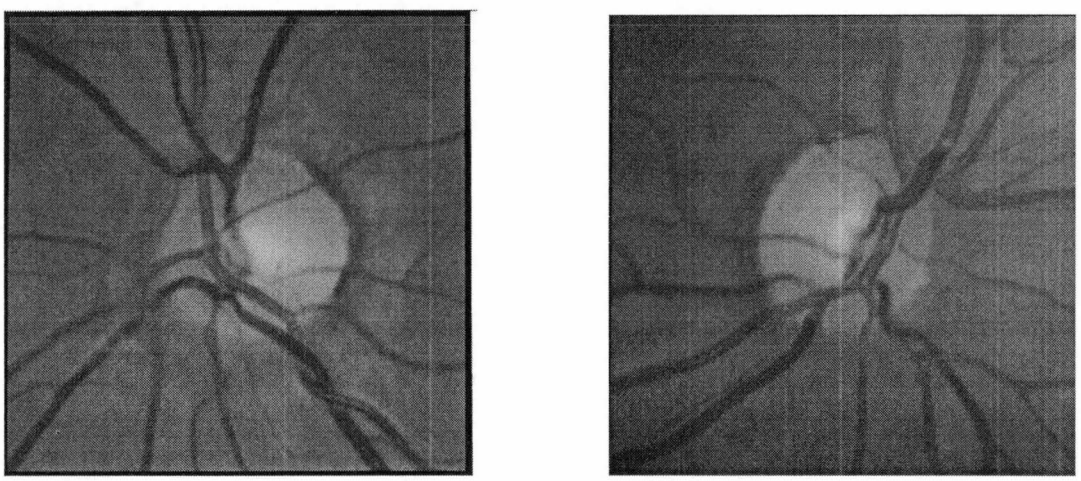


Figure IV.4. Photographs of normal optic disc from study.

Stereoscopic optic disc photographs were taken using a Nidek 3-Dx/F fundus camera (Nidek Co. Ltd, Japan) and Kodachrome ISO 64 film processed by Kodak (Eastman Kodak Co, Rochester, NY) **(Figure IV.5)**. Each participant had bilateral 30 degrees color retinal stereophotographs taken centred on the optic disc and macula. 35 mm slide transparencies were mounted in clear plastic sheets, allowing close apposition of stereo pairs.

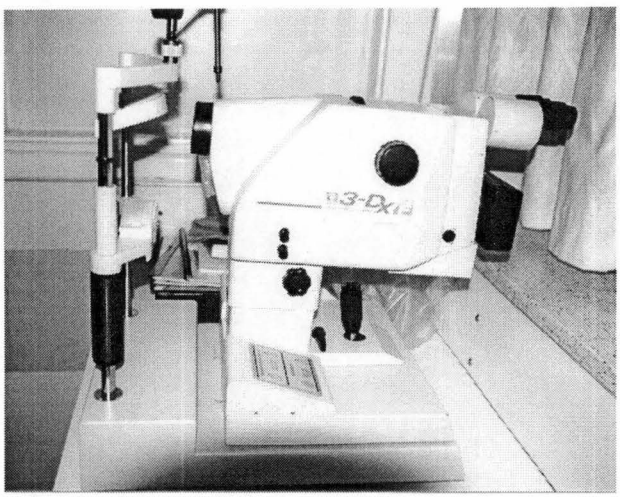


Figure IV.5. Fundus camera from study.

Optic discs are measured from stereophotos using a Pentax stereoviewer II (Asahi Optical Co. Ltd, Japan). All optic discs or high-quality stereophotographs of the discs were scored independently by at least two glaucoma specialists based upon the GIST score protocol set out in **Appendix E** (Coote 1996). If there was a disagreement, a consensus between the ophthalmologists was reached.

Visual field testing was performed with a standard Humphrey automated perimeter (Humphrey, Inc, San Leandro, CA) using a 24-2 array, a size III target, and full threshold test system **(FigureIV.6)**. Both eyes were tested consecutively with a short break between each eye and using the appropriate near correction for 1/3 metre. Testing was monitored by trained staff present in the room. Results were reviewed for reliability using fixation losses, false-positive errors, false-negative errors and short-term fluctuations, and defects were

detected using pattern deviation analysis **(Figure IV.7; FigureIV.8),** with the criteria set out in **Appendix E,** as it standardises for overall decrease in contrast sensitivity which may occur secondary to cataracts (Coote 1996).

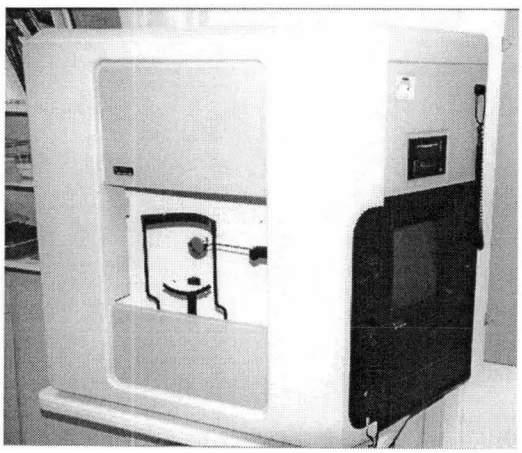
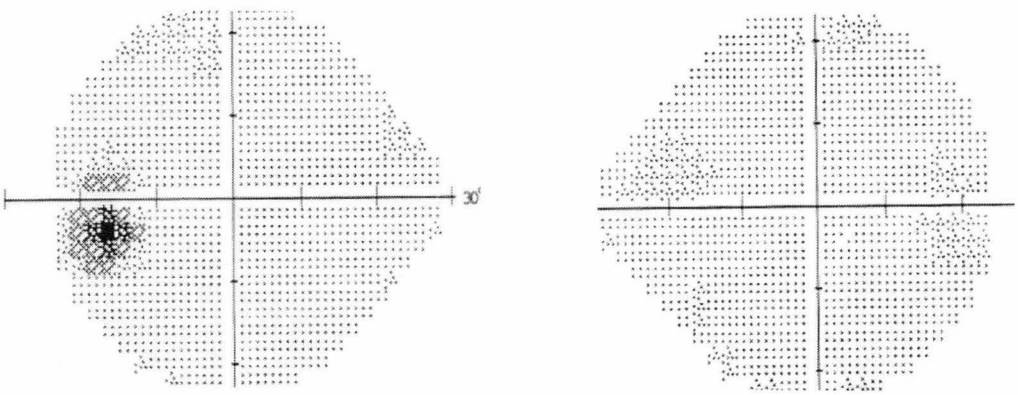


Figure IV.6. Humphrey automated perimeter from study.



Left eye **Right eye**

Figure IV.7. Visual fields of an unaffected subject

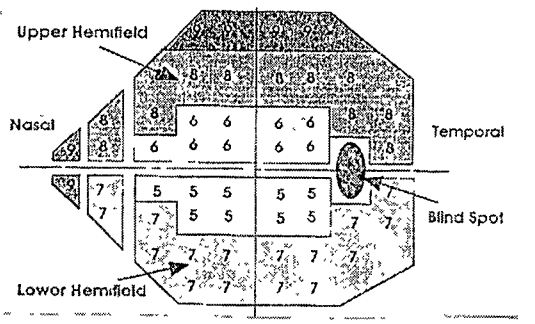


Figure IV.8. Visual field pattern deviation analysis for calculation of GIST score.

IV.III *Glaucoma Definition and GIST Score*

In this study, POAG was defined as an optic neuropathy with a GIST score of 0.7 or higher, with some or all of the following characteristics:

- optic nerve head excavation with thinning of the neuroretinal rim, often with 'Drance' type nerve fibre layer haemorrhages, notching or pitting or significant focal or general loss of retinal fibre layer
- elevation of IOP over a population-based normal or over the average of the unaffected individuals of the pedigree
- visual field defects consistent with the disc changes and with common descriptions of glaucomatous field loss
- no evidence of other forms of glaucoma, trauma, inflammation, pseudoexfoliation of the lens capsule, pigment dispersion, angle dysgenesis or other significant anterior segment pathology, or of potentially occludable angles on gonioscopy (Coote 1996; Damji 1999).

Individuals with a GIST score of 0.5 or lower constituted the 'control' group of unaffected subjects. Those with GIST score of 0.6 formed the 'borderline' group or glaucoma 'suspects'.

The GIST score was developed to give a probability score of glaucoma to family members as it reflects objective physician grading of both patients and family members at the time of examination.

In brief, it assigns a value to the findings of each of the 3 standard tests described above. There are possible 2 points for glaucomatous disc changes, 1 point for elevated IOP and 1 point for visual field changes consistent with glaucoma. These summate to a 'raw score', which is then translated into the pedigree probability or the GIST score (includes a component of probability of unaffected status) as it increases at intervals of 0.1 by each 'raw score' point to a maximum of one. This GIST score is a numerical value between zero and one starting at 0.5, where zero or 0.1 is clinical certainty of absence of disease and 0.9 or one is the definitive diagnosis of POAG (the full methodology and variations of the GIST scoring system are described in the Reference).

Therefore, to a limited extent, the GIST score correlates positively with the severity level of glaucoma in a given individual.

The GIST score excludes those who are normal at examination but are too young to exclude the disease and those with glaucoma that is considered to be highly distinct from the pedigree phenotype. For example, if the 'raw score' is zero, the GIST score is decreased by units of 0.1 depending on age of the individual and the average age of onset of the disease in that pedigree (Coote, 1996).

The GIST score is developed for the individual, and the eye with the highest raw score is used in the calculation. This pedigree probability is not a true probability, which would also need to account for the random occurrence of glaucoma (approximately 3% in Australia) (Mitchell 1996) as well as the reduced probability of second- and third-degree relatives being affected. The GIST score is designed so that patients are given a probability score, rather than included or excluded based on the presence of a single feature. For example, it is possible for an individual to have a GIST score of 0.9 in the absence of elevated IOP.

For 'triangulation', the accuracy of individuals' recall of their family history of glaucoma in questionnaire and at interview was assessed by comparing to the genealogic data and by using the standard GIST score system, which resulted in 84 subjects being reallocated from the familial group to the sporadic group as they had no other affected relatives on examination.

DNA was tested using standard protocol at University of Iowa, USA (Dickinson, 2001), where mutations of the GLC1A gene was ascertained by single-strand conformation polymorphism (SSCP) analysis and subsequent direct sequencing.

IV.IV *Data Management & Statistical Analysis*

All data were entered into a password-verified Microsoft Access database, using automatic skips and range checks. Microsoft Excel was used for tabulations and graphics. Statistical analyses, including chi-square test and multiple logistic regression analyses, were performed using the SPSS statistical package version 10 (SPSS Inc., Chicago, USA). For every subject in the study, data were provided on the following variables:

Group	Control, Familial Glaucoma, Sporadic Glaucoma.
GIST Score	Treated as scaled variable in comparisons of Familial and Sporadic groups.
Degree of Relatives	Levels of genetic connection to glaucoma sufferers (Familial group only)
Pedigree	Genetically related groups
Age	Approximate age in years (Given the study runs over a 4 years period, the best approximation to age is provided by the number of years from date of birth to middle point of data collection period
Gender	Female, male.
IOPmax	maximum intraocular pressure.
Risk variables	Hypertension, smoking, diabetes, transfusion, atherosclerosis, cold extremities, thyroid, migraine, steriods all binary variables with categories TREU/FALSE

Table IV.2 – Variables in the study and their descriptions.

The size of familial glaucoma pedigree groups varied from 2 to 29. There were three groups containing 20-29 members, three groups containing 10-19 members, and 301 groups with fewer than 10 members. Within pedigree comparisons versus sporadic group was confined to the three groups with at least 20 members. Presuming that pedigree groups are imprecise indicators of individual genes or gene groups that may have an association with glaucoma, it was decided that a fruitful path to follow, if practical, would be classification based on genes or genetic groups.

The data were stratified by GIST scores in the familial and sporadic glaucoma groups, and by closest degrees of relatives with POAG in the familial glaucoma group. First-degree relatives are father, mother, son, daughter, and siblings. Second-degree relatives are grandparents, grandson, granddaughter, aunt, uncle, nephew and niece. Third-degree relatives are first cousins, great-grandparents, great aunt/uncle, great-grandson, great-granddaughter. Fourth-degree relatives are more distant relatives, including second cousins' children and great-great-grandparents.

"Degree" of relationship to known glaucoma sufferers was identified on a four level categorisation. The distribution of familial subjects among the four degree classes is as follows:

Table IV.3 – Distribution of degree of relatives in the familial glaucoma group.

1	2	3	4
65%	9%	9%	16%

IV.V *Statistical Methods*

Logistic regression analysis was employed to compare odds for risk factors between the familial and sporadic groups and then adjusted for differences in supplementary variables – age and gender – as appropriate. Where sample sizes were sufficiently large, separate analyses were performed within pedigree groups. Interaction between GIST score and risk factors was included in some analyses to determine if differences between familial and sporadic groups were consistent across different severity levels of glaucoma. The "degree" variable was transformed into a binary variable to allow comparison of degree 1 versus the rest or degrees 1 and 2 versus degrees 3 and 4, and included in an interaction term with risk factors

to establish if differences in odds between the familial and sporadic for any risk factors varied with degree of genetic connection to known glaucoma sufferers. Stepwise logistic regression analysis was employed to determine relations that may exist amongst the risk factors in so far as they affect POAG. Odds ratio estimates are accompanied by 95% confidence limits.

The sampling procedure employed also allowed another perspective to be examined, namely to consider risk factors as response variables. This approach was used to plot odds of true/false in risk variables versus GIST score with separate plots for familial and sporadic groups. It was a useful tool to highlight differences between the familial and sporadic groups where the above analyses indicate significant differences occur.

For each individual risk factor, a log-linear model was fitted and a Likelihood ratio test employed to establish if there were differences in odds among the three populations – control, familial glaucoma, and sporadic glaucoma.

Poisson regression analysis was used to test for the hypothesis of an inverse relationship between decreasing number or proportion of subjects in either the familial or sporadic glaucoma group and increasing GIST scores over time.

To determine if the distribution of GIST scores differed between the familial and sporadic groups, the two-tailed Kolmogorov-Smirnov test was employed (this test is preferred to

the Mann-Whitney test because it is sensitive to any type of difference, including two frequency distributions).

CHAPTER V

RESULTS, STATISTICAL ANALYSIS & DISCUSSION - FAMILIAL AND SPORADIC GLAUCOMA GROUPS CHARACTERISTICS

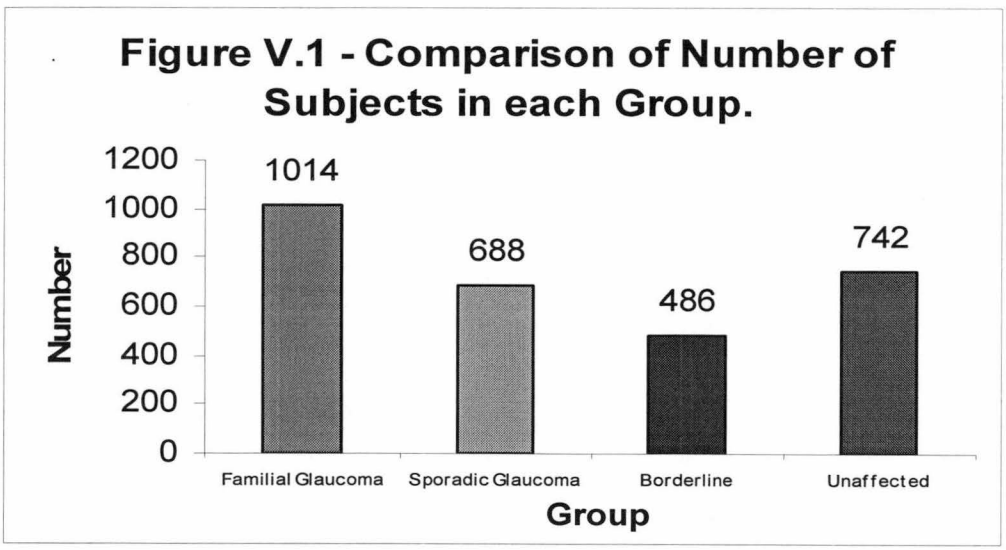
"Data isn't information, information isn't knowledge and knowledge isn't wisdom"

(Anon)

V.I *Group Characteristics*

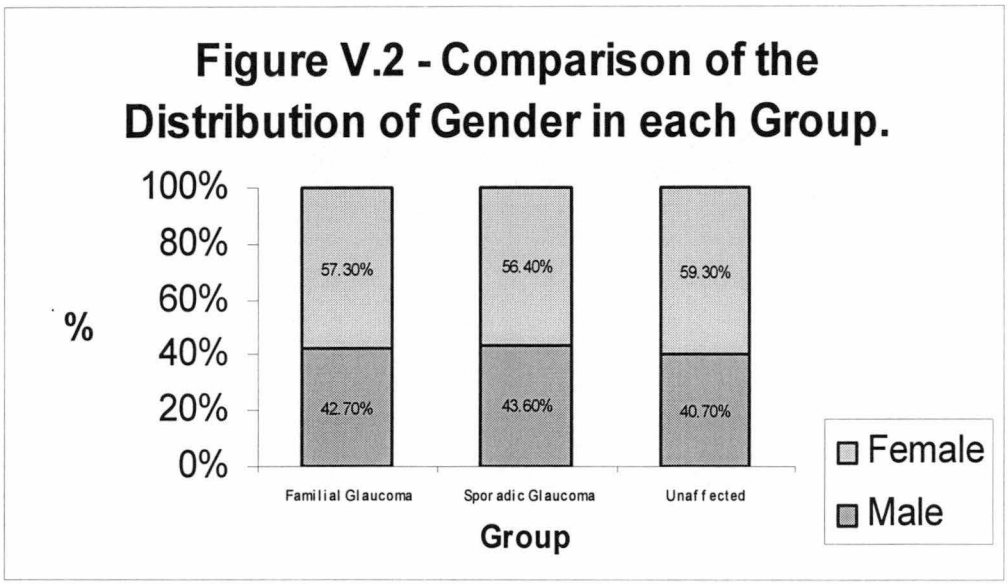
Number of Subjects

Ignoring the borderline or glaucoma 'suspect' group with GIST scores of 0.6 (n=486), 2444 subjects were divided into three groups: familial glaucoma (n=1014), sporadic glaucoma (n=688) and unaffected group (n=742) (**Figure V.1**).



Gender

The distribution of gender in each group was similar (**Figure V.2**). There is no significant difference in gender distribution in the familial glaucoma group compared to the sporadic glaucoma group (OR 1.053; 95% CI 0.819-1.353).



GIST Scores

The percentage of patients in each GIST score group tends to decline with increasing GIST scores in a non-linear fashion in both the familial ($p<0.001$) and sporadic ($p<0.001$) glaucoma groups (**Figures V.3 & V.4**), suggesting a greater percentage of POAG subjects in the lower end of the 'severity' spectrum.

Figure V.3 - Distribution of GIST Scores in the Familial Glaucoma Group.

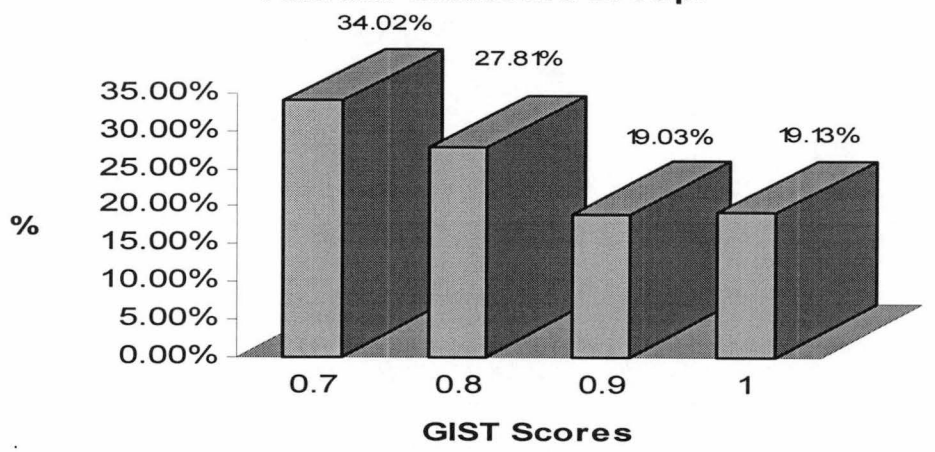


Figure V.4 - Distribution of GIST Scores in the Sporadic Glaucoma Group.

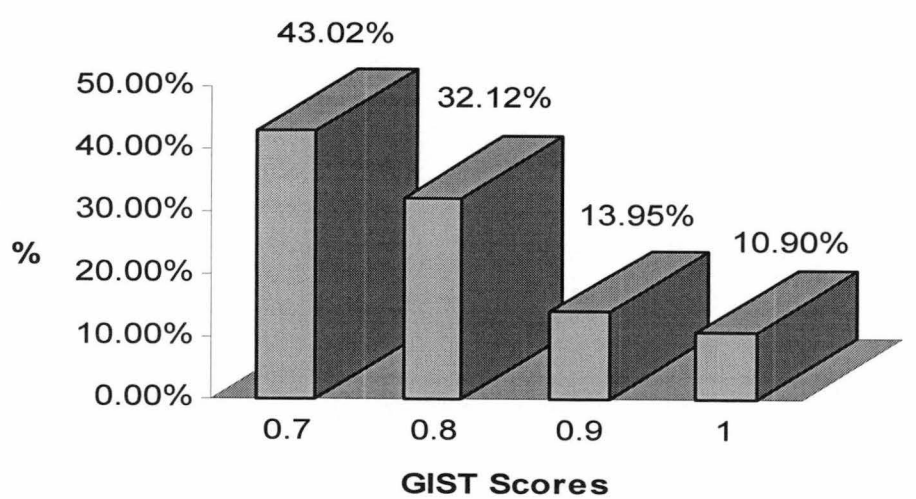


Table V.1 highlights that the large majority (96%) of people in the unaffected group had unchanged GIST scores of 0.5, with the remaining 4% representing those who were expected to have lower GIST scores. The familial glaucoma group has a greater proportion of subjects with a GIST score of 0.9 ($p=0.004$) or 1.0 ($p<0.001$) compared to the sporadic glaucoma group.

Table V.1 – Distribution of the GIST Scores in the familial glaucoma, sporadic glaucoma and unaffected groups.

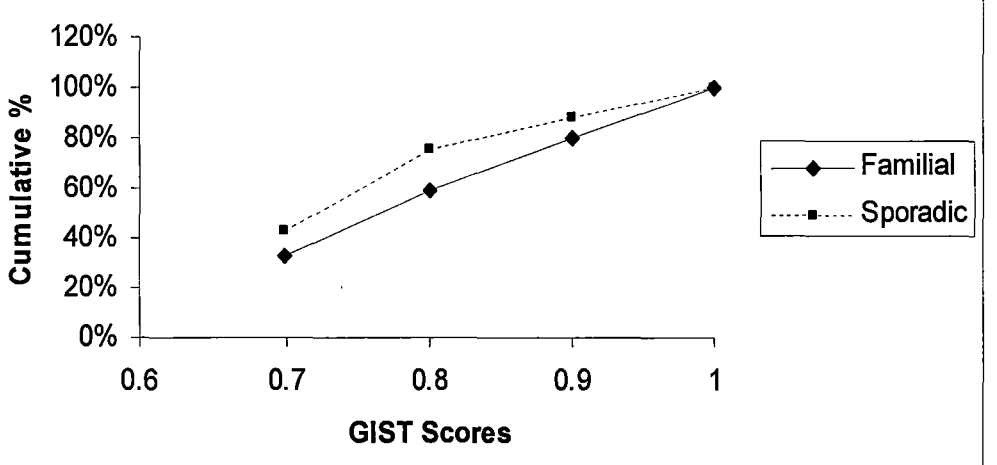
Number (%)	GIST Scores						
Group	**0**	**0.5**	**0.7**	**0.8**	**0.9**	**1**	**Total**
Unaffected	28 (4%)	714 (96%)					742 (100%)
Familial			345 (34%)	282 (28%)	193 (19%)	194 (19%)	1014(100%)
Sporadic			296 (43%)	221 (32%)	96 (14%)	75 (11%)	688 (100%)
Total	28	714	641	503	289	269	2444

There is evidence that the distribution of GIST scores differ between the two glaucoma populations.

The familial glaucoma group is more represented in the higher GIST score levels and the sporadic glaucoma group has a higher prevalence in the lower GIST score levels. 38.2% of the familial glaucoma group compared to 24.9% of the sporadic glaucoma group has a GIST score of 0.9 or 1.0 ($p<0.005$).

The cumulative graph of GIST scores (**Figure V.5**) highlights the discrepancy in its distribution within the familial glaucoma group compared to the sporadic glaucoma group, which is highly statistically significant in the 2-tailed Kolmogorov-Smirnov test ($p<0.001$).

Figure V.5 - Cumulative Graph of GIST Scores in Familial and Sporadic Glaucomas



Degree of Relatives

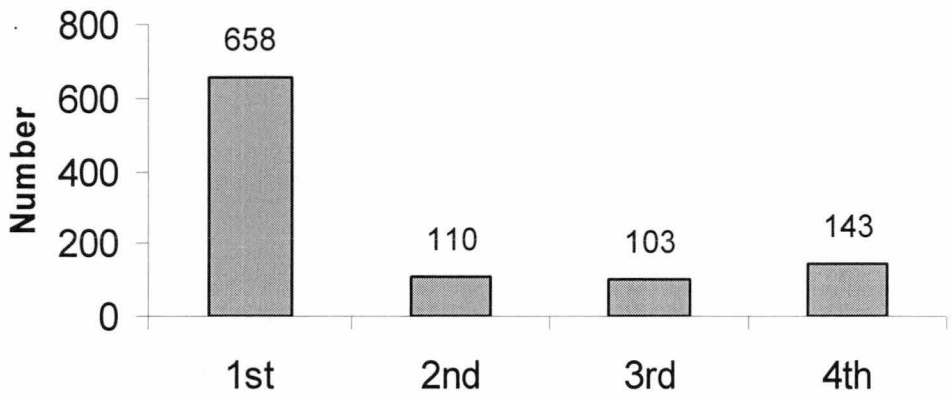
Table V.2 – Distribution of the closest degree of relatives affected with POAG in the familial glaucoma and sporadic glaucoma groups.

Number (%)	Degree of Relatives					
Group	**1**	**2**	**3**	**4**	**Unrelated**	**Total**
Unaffected					742 (100%)	742
Familial	658 (65%)	110 (11%)	103 (10%)	143 (14%)		1014
Sporadic					688 (100%)	688
Total	658	110	103	143	1430	2444

Table V.2 illustrates that in the familial glaucoma group, nearly two-thirds of people had a first-degree relative also affected with POAG. Nearly a sixth of the familial glaucoma group had a fourth-degree or more distant relative also affected with POAG. Approximately 10% each had a second- or third-degree affected relative (**Figure V.6**).

By definition, no-one in the sporadic glaucoma group had an affected relative.

Figure V.6 - Distribution of closest Degree of Relatives affected with POAG in the Familial Glaucoma Group.



V.II *Potential Confounding Effect of Age and Gender*

Possible relationships among supplementary variables such as age and gender may exist and are presumed to be important in explaining odds of a person having glaucoma and for which adjustment is required in the examination of relationships with potential risk factors. **Figure V.7** illustrates the median age and standard deviation in each group, which appeared to be similar between familial and sporadic glaucoma. Both groups consisted of older individuals compared to the unaffected group with GIST score of 0.5 or less (median age for females and males was 54 and 53 years, respectively).

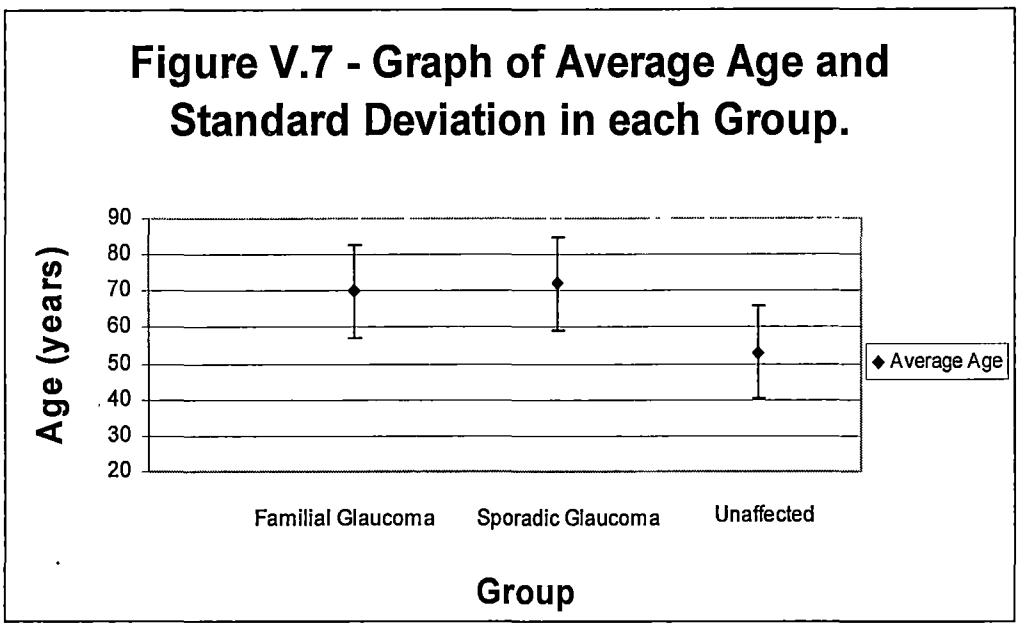


Table V.3 – Median age of males and females in familial glaucoma, sporadic glaucoma and unaffected groups.

Median Age (Year)	Gender	
Group	**F**	**M**
Unaffected	54	53
Familial	71	69
Sporadic	73	71
Grand Total	66	65

The situation becomes clearer when the age difference between the groups are broken down by GIST scores:

Table V.4 – Median age in familial glaucoma, sporadic glaucoma and unaffected groups at different GIST scores.

Median Age (Year)	GIST Score						
Group	**0**	**0.5**	**0.7**	**0.8**	**0.9**	**1**	**Total**
Unaffected	55	53					53
Familial			65	71	72	74	70
Sporadic			71	73	74	74	72
Total	55	53	68	72	73	74	66

It can be seen that the aberrant group is the familial group with GIST levels of 0.7. Breaking the figures in the above cells into female and male components (not shown), there is very

little gender imbalance except in the familial group with GIST score of 0.7, where it is found that the median female age is 66 and the median male age is 64. For all other cells the median age for females and males ranges between 71 and 75 years.

The influence of *age* on the relative odds for the familial and sporadic groups is seen in the following test results in which *age* is the single explanatory variable in the model:

Table V.5 – Comparison of odds ratio and confidence intervals with age as a single explanatory variable.

GIST scores included	p-value	odds ratio	95% confidence interval
0.7 to 1.0	<0.001	1.016	1.008 – 1.024
0.8 to 1.0	0.14	1.008	0.997 – 1.019

There is no significant difference between familial and sporadic glaucoma groups based on subjects with GIST scores of 0.8 or higher (OR 1.002; 95%CI 0.991-1.013).

For *gender* there is not a significant difference.

V.III *Comparisons within pedigree groups*

Table V.6 shows the distribution of 87 subjects in pedigree GTas01, the second largest pedigree in the study.

Table V.6 – Distribution of subjects by gender and GIST scores in the GTas01 pedigree.

Number		**GIST SCORE**						
Group	**Gender**	**0**	**0.5**	**0.7**	**0.8**	**0.9**	**1.0**	**Total**
Unaffected	F	1	34					35
	M		27					27
Unaffected Total		**1**	**61**					**62**
Familial	F			8	3		3	14
	M			3	6	1	1	11
Familial Total				**11**	**9**	**1**	**4**	**25**
Total		**1**	**61**	**11**	**9**	**1**	**4**	**87**

These numbers are too small to allow separation of effects of risk factors from GIST scores.

Using GTas02 as an example below, the same situation applies with other pedigree groups which have smaller number of family members.

If the members with GIST = 0.7 or greater are included there are only 20 members of GTas02.

Table V.7 – Distribution of subjects with GIST score 0.7 or greater in GTas02 pedigree.

Group	Number (%)
Familial GTas02	20 (2.8%)
Sporadic	688 (97 2%)
Total	708 (100%)

If the restriction is for GIST of 0.8 or greater there are only 13 members in GTas02.

Table V.8 – Distribution of subjects with GIST score 0.8 or greater in GTas02 pedigree.

Group	Number (%)
Familial GTas02	13 (3.2%)
Sporadic	392 (96.8%)
Total	405 (100%)

Indeed, the numbers are too small for meaningful analysis, given that the 13 subjects in GTas02 have to be split between the two levels (true/false) of a risk factor.

V.IV *Comparing Odds for Familial versus Sporadic Glaucoma Groups*

Table V.9 and **Figure V.8** summarises the unadjusted odds ratios (familial versus sporadic glaucoma) and 95% confidence intervals for various risk factors.

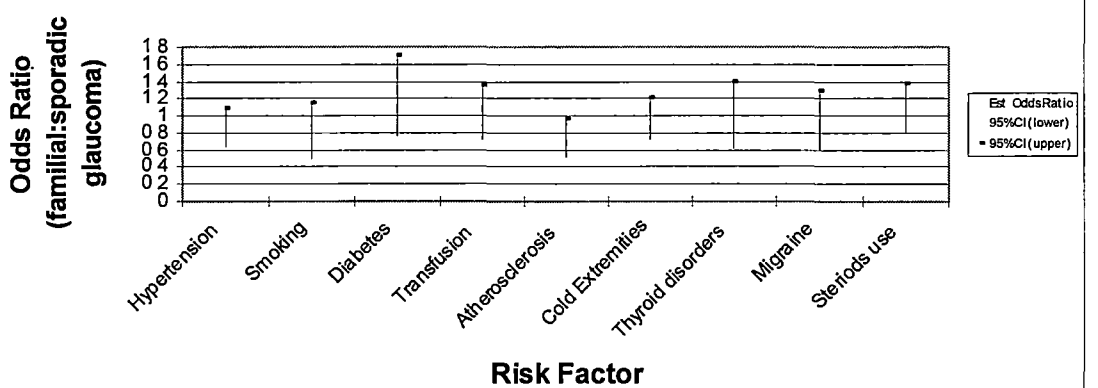
Table V.9 – Unadjusted odds ratios and 95% confidence intervals for various risk factors present in familial glaucoma versus sporadic glaucoma groups.

Risk factor	p-value	odds ratio	95% confidence interval
Hypertension	0.19	0.838	0.643 – 1.092
Smoking	0.19	0.752	0.491 – 1.152
Diabetes	0.5	1.138	0.767 – 1.688
Transfusion	0.96	0.992	0.726 – 1.355
Atherosclerosis	0.03	0.699	0.505 – 0.967
Cold extremities	0.5	0.920	0.709 – 1.201
Thyroid	0.7	0.916	0.607 – 1.382
Migraine	0.5	0.879	0.599 – 1.291
Steroids	0.75	1.045	0.799 – 1.366

Only *atherosclerosis* (P=0.03) was found to be significant in the unadjusted odds ratios.

In stepwise fitting employing all risk factors, no risk factor is retained in the final equation (these results are elaborated in chapters VI-XVI).

Figure V.8 - Graph of Unadjusted Odds Ratio for various Risk Factors.



DISCUSSION

Age and Gender

1. Comparisons between familial and sporadic groups should be restricted to subjects with GIST scores greater than 0.7 if confounding effects of age and gender are to be minimised.

2. Any comparisons between the familial or sporadic groups and control group will be confounded with both age and gender.

The following **assumptions** have been made in the study:

1. the relative proportions provide unbiased estimates of the distribution of the Tasmanian population who have GIST scores greater than or equal to 0.5 among the three subpopulations; and
2. the samples from the three populations can be treated as though they are randomly selected from the respective populations, although many of the unaffected are relatives of the affected participants.

In this study, nearly 60% of all glaucoma subjects were objectively classified into the familial group (1014/1702 or 59.6%), giving support to previous studies which reported that over 50% of all people with POAG in the community have a positive family history of glaucoma (McNaught 2000).

This investigation also found that the distribution of gender between the familial and sporadic glaucoma groups was similar and not statistically significant (OR 1.053; 95% CI 0.819-1.353), reflecting the autosomal rather than sex-linked inheritance of familial POAG. In contrast, there were a greater proportion of females (43.1%) in the total glaucoma group compared to that in the unaffected 'control' group (40.7%), but the difference was not statistically significant (OR 1.102; 95% CI 0.925 – 1.313).

This supports the Blue Mountains Eye Study, which found a higher prevalence of POAG in Australian women in comparison to men (OR 1.5; 95%CI 1.0-2.2)(Mitchell 1996). The discrepancy in magnitude of gender difference may reflect true differences in the two study populations or may reflect a chance variation. In contrast, the Melbourne Visual Impairment Project found no relationship between POAG and gender (Wensor 1998). Notably, there was a higher rate in white men in some studies (Leibowitz 1980; Leske 1996), but not in others (Mason 1989; Tielsch 1991; Leske 1983; Kahn 1980; Hollows 1966; Klein 1991; Alward 2000). Indeed, population-based studies have yielded conflicting results on sex-specific glaucoma prevalence.

In both the familial ($p<0.001$) and sporadic ($p<0.001$) glaucoma groups, the proportion of subjects in each GIST score group tends to decline in a non-linear fashion with increasing GIST scores This may reflect the co-morbidities associated with more severe glaucoma types. It may also simply reflect the age-glaucoma relationship, whereby glaucoma becomes more prevalent and severe with increasing age, as do other medical illnesses that are associated with higher mortality, such as coronary artery disease and cancer.

In the Rotterdam eye study, siblings of patients with POAG (familial POAG) were on average 3 years younger than siblings of people with no glaucoma and had significantly higher IOP and cup-to-disc ratios (Wolfs 1998). In this study, the distribution of GIST scores (probability scores of glaucoma based on IOP, optic disc and visual field changes) tended to be skewed towards higher range of scores in the familial glaucoma group compared to the sporadic glaucoma group ($p<0.001$), which may reflect an earlier onset and/or higher severity of glaucoma in the familial group. Indeed, familial POAG pedigrees with the Gln368STOP mutation in the myocilin gene is of a younger age of onset, has a higher peak IOP and is more likely to have undergone glaucoma drainage surgery compared to non-mutation glaucoma cases (Craig 2001). Furthermore, some of the other mutations found in the myocilin gene, such as Thr377met, Tyr437His and Ile477Asn, have an even younger average age of onset, higher peak IOP and is more likely to have undergone glaucoma drainage surgery (Mackey in-press, Alward 1998).

Further elaboration is required to investigate the hypothesis of an earlier onset and/or higher severity of glaucoma in the familial group..

The small number of subjects within each pedigree precludes valid statistical comparisons of odds of clinical risk factors in each pedigree of familial glaucoma versus the sporadic glaucoma group. The most useful approach in relation to pedigree groups would seem to be a search for indicator genes that would allow combination of pedigrees with a common gene, so that sample numbers are built to a stage where reliable analysis is possible and the

separation of possible genetic effects from age and severity is possible. This will be discussed further in chapter VI.

It is thus clear that both age and gender are important potential confounders and should be adjusted for in our study comparing the prevalence of various risk factors between the familial and sporadic glaucoma groups.

Clinical Risk Factors

The preliminary unadjusted odds ratios suggest that atherosclerosis is significantly less prevalent in the familial glaucoma group compared to the sporadic glaucoma group. However, after stepwise fitting of all risk factors, it is not retained in the final equation, indicating that the effect of atherosclerosis may be exerted by a third factor such as hypertension. These relationships will be examined in detail in chapters VI-XI.

CHAPTER VI

RESULTS, STATISTICAL ANALYSIS & DISCUSSION – HYPERTENSION

RESULTS

Analysis was undertaken from two perspectives: firstly, how the odds of a subject having hypertension differed between the familial and sporadic groups and secondly, how the odds of having hypertension differed as the GIST score changed. Finally, there was a test to determine whether the relationship between the odds of having hypertension *versus* GIST score varied between the familial and sporadic groups.

VI.I *Familial versus Sporadic Glaucoma Comparison*

The following results in **Table VI.1** apply when the analysis is restricted to subjects with GIST scores greater than 0.7.

Table VI.1 – Unadjusted and adjusted odds ratios and confidence intervals of hypertension in familial glaucoma group compared to sporadic glaucoma group.

Adjustment	p-value	odds ratio	95% confidence interval
None	0.19	0.84	0.64 – 1.09
All other risk factors	0.28	0.86	0.65 – 1.13
GIST Score	0.16	0.82	0.63 – 1.08
Age and gender	0.17	0.83	0.63 – 1.08

Initial analysis showed that the prevalence of hypertension is not statistically significant in the familial glaucoma group compared to the sporadic glaucoma group (unadjusted OR 0.84; 95%CI 0.64-1.09).

The difference is unchanged after adjusting for all other risk factors (diabetes, smoking, migraine, atherosclerosis, steroids, transfusion, cold extremities and thyroid disorders) (OR 0.86; 95%CI 0.65-1.13), or age and gender (OR 0.83; 95%CI 0.63-1.08).

VI.II *Relationship of Odds of having Hypertension versus GIST Score*

As shown in **table VI.1**, the odds of hypertension (familial versus sporadic glaucoma) strengthened marginally but were not significant after adjusting for GIST scores (OR 0.82; 95%CI 0.63-1.08).

The distribution of subjects across GIST scores is summarised in **table VI.2** below.

Table VI.2 – Distribution of hypertension versus no hypertension and odds of hypertension as a function of GIST scores in the familial glaucoma, sporadic glaucoma and unaffected groups.

Number		GIST Score						
HYPERTENSION	**Group**	**0**	**0.5**	**0.7**	**0.8**	**0.9**	**1**	**Total**
FALSE	Unaffected	20	551					571
	Familial			211	181	123	127	642
	Sporadic			182	143	74	51	450
FALSE Total		**20**	**551**	**393**	**324**	**197**	**178**	**1663**
TRUE	Unaffected	8	163					171
	Familial			134	101	70	67	372
	Sporadic			114	78	22	24	238
TRUE Total		**8**	**163**	**248**	**179**	**92**	**91**	**781**
Grand Total		**28**	**714**	**641**	**503**	**289**	**269**	**2444**

There is a greater number of people with no hypertension compared to people with hypertension across all GIST scores in all 3 groups. A total of 238 out of 688 (34.6%) in the sporadic glaucoma group has hypertension, while a total of 372 out of 1014 (36.7%) in the familial glaucoma group has hypertension (**Table VI.2**). Overall, a total of 610 glaucoma patients out of 1702 (35.8%) have hypertension.

Figure VI.1 below plots the odds of a subject having hypertension as a function of GIST score. Separate plots are shown for the familial and sporadic groups and the average of the two groups. Please note that the value provided at a GIST score of 0.5 is obtained from the unaffected group.

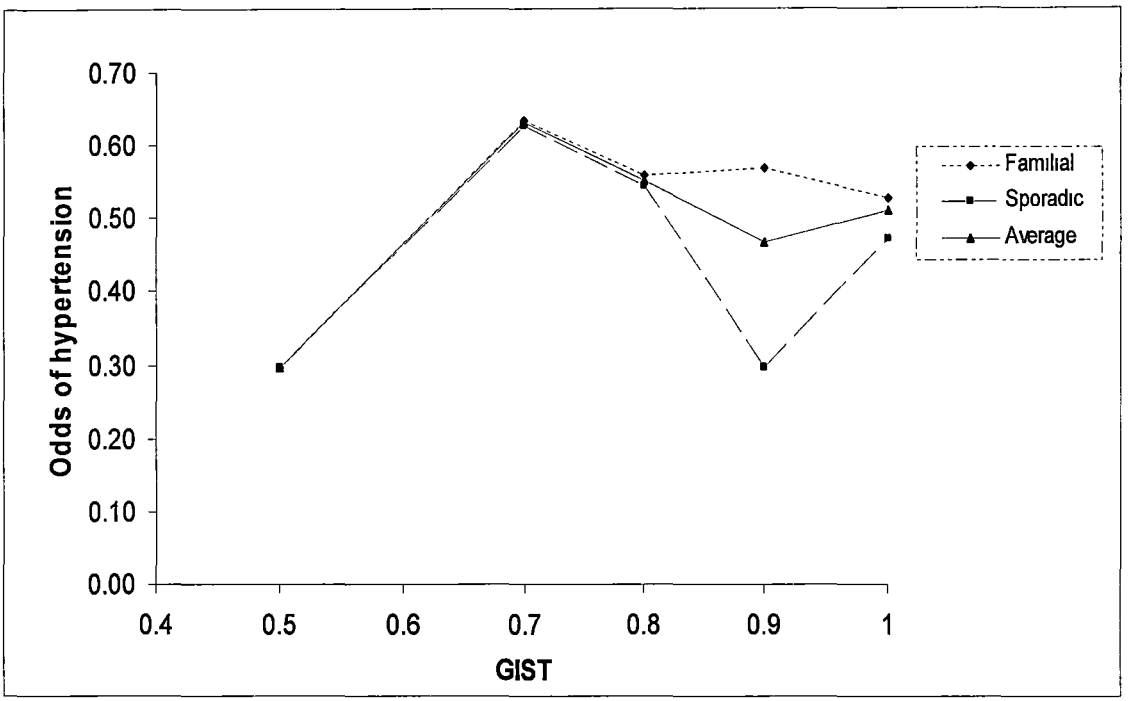


Figure VI – Comparison of odds of hypertension in familial glaucoma, sporadic glaucoma and unaffected groups

It can be seen in Figure VI above, there is a significant difference in odds for familial and sporadic groups when the GIST score is 0.8 or greater and that the observed difference cannot be explained as an age or a gender effect. It appears that the odds of hypertension remains constant in the familial group, but reduces in the sporadic group in the range from 0.8 to 1.0. It should be noted that the comparison at GIST =0.7 is confounded by age and gender (chapter V).

VI.III *Relationship of Odds of Hypertension versus Gender*

Table VI.3 shows the number of males and females with and without hypertension in all three groups.

Table VI.3 – Distribution of hypertension versus no hypertension in males and females in familial glaucoma, sporadic glaucoma and unaffected groups.

Count of Type		Gender		
HYPERTENSION	**Type**	**F**	**M**	**Total**
TRUE	Control	117	54	171
	Familial	240	132	372
	Sporadic	154	84	238
TRUE Total		**511**	**270**	**781**
FALSE	Control	323	248	571
	Familial	341	301	642
	Sporadic	234	216	450
FALSE Total		**898**	**765**	**1663**
Grand Total		**1409**	**1035**	**2444**

In the familial glaucoma group, hypertension was found in 240 out of 581 females (41.3%) and in 132 out of 433 males (30.5%). In the sporadic glaucoma group, hypertension was found in 154 out of 388 females (39.7%) and in 84 out of 300 males (28.0%). These differences were not statistically significant (OR 1.01; 95% CI 0.72 – 1.40). Nonetheless, adjusting for age and gender marginally improved the significance of the hypertension difference between familial and sporadic glaucoma groups from odds ratio of 0.84 to 0.83.

VI.IV *Relationship to Degree of Relatives*

Restricting the familial group dependent on degree of relationship produced the following results in comparison of the familial and sporadic groups using subjects with GIST scores of 0.8 or higher:

Table VI.4 – Odds of hypertension by degree of relatives of familial glaucoma group compared to the sporadic glaucoma group.

Degree of Relative	p-value	odds ratio	95% confidence intervals
1st	0.555	0.916	0.684 – 1.227
2nd	0.229	0.727	0.433 – 1.222
3rd	0.324	0.765	0.450 – 1.302
4th	0.099	0.683	0.433 – 1.075

There is no statistically significant difference in odds of hypertension between the familial glaucoma degree of relatives subgroups and sporadic glaucoma, suggesting the absence of any difference overall.

VI.V – *Relationship to Mutations in GLC1A Gene*

Table VI.5 summarises the prevalence of hypertension and no hypertension in the familial glaucoma GTas02 pedigree as a function of laboratory-identified Gln368STOP mutation in the GLC1A gene.

Table VI.5 – 2x2 table comparing the prevalence of hypertension and GLC1A gene Gln608STOP mutation in the familial glaucoma GTas02 pedigree.

Hypertension	GLC1A mutation		
	Yes	No	Total
Yes	7	12	19
No	36	92	128
TOTAL	43	104	147

Note - There were an extra 8 cases where the gene test results are ambiguous and hence were excluded from the above figures.

Application of the Chi-squared test to the above data yielded a p-value of 0.4, which suggests that the observed difference in the ratio of hypertension to no hypertension cases between the GLC1A gene mutation and no mutation groups could reasonably be explained as a chance variation.

DISCUSSION

There appears to be no statistically significant difference in the odds of hypertension between the familial and sporadic glaucoma groups in subjects with GIST scores of 0.8 or greater after adjusting for other clinical factors (OR 0.86; 95%CI 0.65-1.13), and differences in age and gender (OR 0.83; 95%CI 0.63-1.08) or GIST scores (OR 0.82; 95%CI 0.63-1.08).

Moreover, there is no statistically significant difference in odds of hypertension between degree of relatives subgroups in familial glaucoma and sporadic glaucoma.

An extension of the study was undertaken to examine the relationship between the ratio of hypertension to non-hypertension cases and presence or absence of known mutations in the GLC1A gene. This gene was identified by standard laboratory tests with single-strand conformation polymorphism (SSCP) analysis and subsequent direct sequencing (Dicksinson 2001) in a specific pedigree, namely GTas02. A chi-square probability of 0.4 suggests that the

difference observed was not statistically significant and was likely to be due to chance. By extrapolation, this implies that there is unlikely to be any statistically significant difference in the prevalence of hypertension between familial and sporadic glaucoma groups.

Indeed, it is likely that hypertension is a multifactorial disorder, with varying degrees of genetic predisposition, and modified by a multitude of environmental/lifestyle factors such as obesity and level of physical activity. A direct association between hypertension and familial POAG would seem to be too simplistic.

The possible role of systemic vascular factors in POAG pathogenesis has been debated for many years (Phelps 1972; Drance 1973). Hypertension could increase POAG risk indirectly, through its association with high IOP (Leske 1983; Hiller 1982; Bulpitt 1975; Bengtsson 1972; Klein 1981), or may increase POAG risk directly through small-vessel disease. That is, ocular capillary and small vessels circulation may be more precarious as blood pressure increases, and impaired perfusion pressure in vessels supplying the optic disc may contribute to glaucoma (Ellenberg 1979). Local vascular factors such as myogenic tone may also play a role (Wilson 1999).

On the other hand, hypertension might 'protect' against POAG by providing an adequate perfusion pressure, which has led to concern that antihypertensive treatment may increase risk of field loss (Phelps 1972; Weinstock 1973). However, this premise is not supported by findings in the Long Island Study (Leske 1996).

Paradoxically, a decrease in blood pressure (relative to IOP), such as in hypotensive crisis, increases glaucoma risk (Drance 1973). This occurrence of glaucomatous damage despite normal or low pressures, supports the view of glaucoma as two separate diseases (high-tension and low-tension glaucoma) with different pathophysiology. A role has been hypothesised for antihypertensive treatment, hypotensive episodes, or any vascular-related factor that could affect the blood pressure-IOP relationship needed for optic disc perfusion (Leske 1983; Kahn 1980).

Results from population-based studies have differed. In the Baltimore Eye Survey, there was no significant association between hypertension and POAG. Race-adjusted odds ratios tended to be lower at younger ages and higher at older ages, suggesting that hypertension is protective at ages under 60 and damaging at older ages (Tielsch 1995). However, the results were not statistically significant.

In the smaller Rotterdam POAG study (n=42), a positive relationship between hypertension and POAG was found only in cases with high IOP; however the odds ratio was not significant (Dielemans 1995). Furthermore, the Barbados Eye Study (n=302 definite cases of POAG) found that an elevated systolic blood pressure, regardless of treatment, was associated with POAG at ages under 70 years; however the association was weak and did not persist after age-sex adjustment or multivariate analysis (Leske 1995). More recently, the Long Island Study also did not support the hypothesis that systemic hypertension independently increases the risk of POAG field defect (Leske 1996).

It is of interest to note that both the Barbados Eye Study and the Long Island study found a significant association between POAG and low perfusion pressure (low diastolic blood pressure-IOP differences). An association between low systolic and diastolic blood pressure to IOP ratios has also been documented (Leske 1995; Leske 1996) but not examined in the present study. The role of vascular risk factors is consistent with findings of low blood pressure to IOP ratios, but this could be explained by the high IOP in POAG rather than by a relatively low blood pressure. Moreover, the relationship between systemic blood pressure and IOP may not accurately reflect the local perfusion pressure at the optic disc.

At present, therefore, there is insufficient evidence to support the role of hypertension as a strong independent risk factor for POAG or its familial subgroup.

CHAPTER VII

RESULTS, STATISTICAL ANALYSIS & DISCUSSION – DIABETES MELLITUS

RESULTS

Analysis was undertaken from two perspectives: firstly, how the odds of a subject being a diabetic differed between the familial and sporadic groups and secondly, how the odds of having diabetes changed as the GIST score changed. Finally, there was a test to determine whether the relationship between the odds of having diabetes versus GIST score varied between the familial and sporadic groups.

VII.I *Familial versus Sporadic Glaucoma Comparison*

Table VII.1 – Unadjusted and adjusted odds ratios and 95% confidence intervals of diabetes mellitus in familial glaucoma group compared to sporadic glaucoma group.

Adjustment	p-value	odds ratio	95% confidence interval
None	0.5	1.14	0.77 – 1.69
Other risk factors	0.3	1.22	0.81 – 1.83
GIST Score	0.7	1.09	0.73 – 1.62
Age and gender	0.5	1.14	0.77 – 1.70

Table VII.1 records the p-value, odds ratio plus 95% confidence limits for the unadjusted and adjusted situations: there is no evidence of differences in odds of diabetes between familial and sporadic groups.

VII.II *Relationship of odds of Diabetes versus GIST score*

The distribution of subjects across GIST scores is summarised in **table VII.2.**

Table VII.2 – Distribution of diabetes versus no diabetes and odds of diabetes as a function of GIST scores in the familial glaucoma, sporadic glaucoma and unaffected groups.

Number		GIST Score						
DIABETES	**Group**	**0**	**0.5**	**0.7**	**0.8**	**0.9**	**1**	**Total**
FALSE	Unaffected	24	694					718
	Familial			309	253	163	183	908
	Sporadic			258	192	86	68	604
FALSE Total		**24**	**694**	**567**	**445**	**249**	**251**	**2230**
TRUE	Unaffected	4	20					24
	Familial			36	29	30	11	106
	Sporadic			38	29	10	7	84
TRUE Total		**4**	**20**	**74**	**58**	**40**	**18**	**214**
Grand Total		**28**	**714**	**641**	**503**	**289**	**269**	**2444**

There was a greater number of people with no diabetes compared to people with diabetes across all GIST scores in all three groups. A total of 76 out of 688 (11.0%) in the sporadic glaucoma group had diabetes, while a total of 160 out of 1014 (15.8%) in the familial glaucoma group had diabetes (**Table VII.2**). Overall, a total of 236 glaucoma patients out of 1702 (13.9%) had diabetes. A test of the hypothesis that the odds of a person having diabetes is the same for the familial and sporadic glaucoma groups is not rejected after adjusting for GIST scores (OR 1.09; 95% CI 0.73-1.62).

Figure VII.1 below plots the odds of a subject having diabetes as a function of GIST score. Separate plots are shown for the familial and sporadic groups and their averages. However, the difference in patterns was not statistically significant. Note that the value provided at a GIST score of 0.5 was obtained from the unaffected 'controls' group.

Pairwise comparisons established that the odds of diabetes at GIST score of 0.5 and 1 were not statistically significant between the two glaucoma populations.

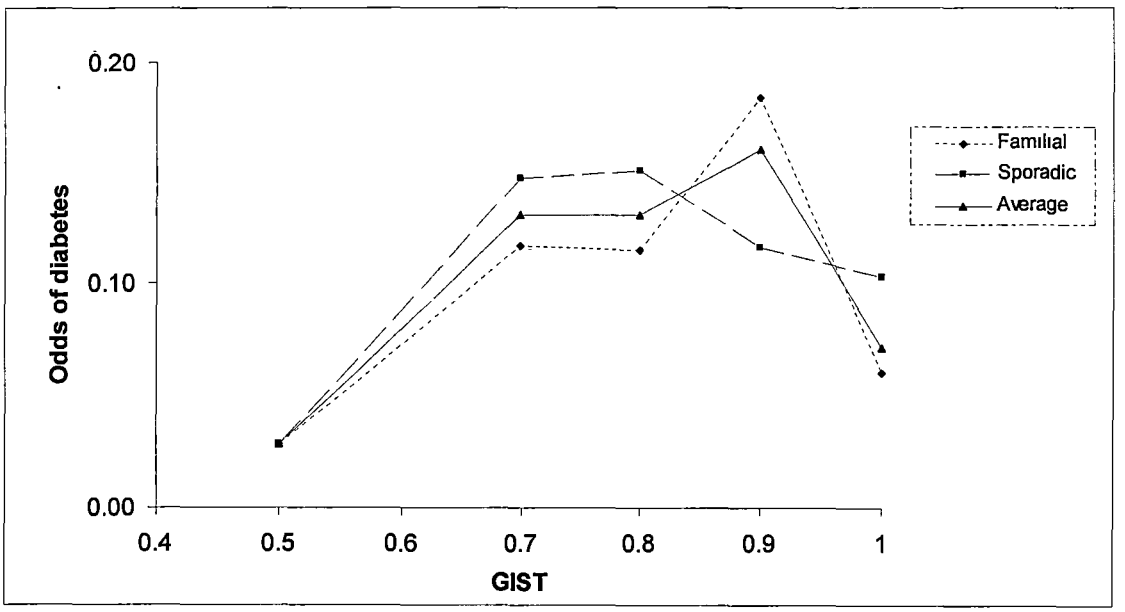


Figure VII – Comparison of odds of diabetes in familial glaucoma, sporadic glaucoma and unaffected groups.

VII.III – *Relationship of Odds of Diabetes versus Gender*

Table VII.3 shows the number of males and females with and without diabetes in all three groups.

Table VII.3 – Distribution of diabetes versus no diabetes in males and females in familial glaucoma, sporadic glaucoma and unaffected groups.

Count of Type		Gender		
DIABETES	**Type**	**F**	**M**	**Total**
TRUE	Control	14	10	24
	Familial	56	50	106
	Sporadic	47	37	84
TRUE Total		**117**	**97**	**214**
FALSE	Control	426	292	718
	Familial	525	383	908
	Sporadic	341	263	604
FALSE Total		**1292**	**938**	**2230**
Grand Total		**1409**	**1035**	**2444**

In the familial glaucoma group, diabetes mellitus was found in 56 out of 581 females (9.6%) and in 50 out of 433 males (11.5%). In the sporadic glaucoma group, diabetes was found in 47 out of 388 females (12.1%) and in 37 out of 300 males (12.3%). The differences were not statistically significant (OR 1.13; 95%CI 0.64-2.1). The odds of diabetes between familial and sporadic glaucoma groups remained non-significant after adjusting for gender and age (OR 1.14; 95%CI 0.77-1.70).

VII.IV – *Relationship to Degree of Relatives*

Table VII.4 – Odds of diabetes in subgroups of familial glaucoma versus sporadic glaucoma group as a function of degrees of relatives affected with POAG.

Degree of Relative	p-value	odds ratio	95% confidence intervals
1st	0.369	1.225	0.787 – 1.907
2nd	0.879	1.064	0.479 – 2.359
3rd	0.519	0.784	0.375 – 1.640
4th	0.648	1 183	0.575 – 2.437

As shown in **Table VII.4**, subgroups of degrees of relatives in the familial glaucoma group demonstrate no significant difference in odds of diabetes between the familial and sporadic glaucoma groups.

DISCUSSION

The prevalence of diabetes amongst individuals with POAG in this study was 13.9%, slightly more than that reported in the Blue Mountains Eye Study (12.5%) (Mitchell 1997). This may be because diabetes was ascertained by questionnaire and by use of antidiabetic medications in this study. The use of biochemical evidence of abnormal glucose tolerance might have detected undiagnosed diabetes in the community and reveal a higher difference.

The lack of significant association between familial glaucoma and diabetes (OR 1.14; 95%CI 0.77-1.69) persisted after adjusting for age and gender (OR 1.14; 95%CI 0.77-1.70), another potential risk factors (OR 1.22; 95%CI 0.81-1.83). This may be due to the fact that the majority of diabetes is non-insulin dependent adult-onset diabetes, which increases with age and environmental factors such as obesity. It would probably have the same influence on both the familial and sporadic glaucoma groups. This finding also supports the conclusion from the Blue Mountains Eye Study, which reported no change in the diabetes-glaucoma relation after adjusting for family history of glaucoma (Mitchell 1997).

Similarly, no significant association was found in the distribution of diabetes between the familial and sporadic glaucoma groups as a function of GIST scores or degree of relatives.

A number of hypotheses on the pathogenesis of an association between diabetes and glaucoma have been proposed. The increased prevalence of glaucoma may reflect optic nerve damage as a result of vascular or other effects of diabetes (Becker, 1971), such as those on the small vessels of the eye (Wilson 1999). The reported IOP rises could be a manifestation of the systemic autonomic dysfunction observed in diabetes (Armstrong 1960; Mapstone 1985). This may provide the mechanism for association not only between diabetes and glaucoma, but also between diabetes and elevated IOP reported in some studies (Tielsch 1995; Leske 1983; Jain 1967; Mitchell 1997) but not in others (Bouzas 1971; Seddon 1983). Moreover, a direct osmotic effect of hyperglycaemia might be expected to lower rather than increase IOP (Dielemans, 1994).

Therefore, at present, there is no strong evidence for an association between familial POAG and diabetes mellitus. However, if there is an association between diabetes and POAG, it may in part depend on the subtype of diabetes mellitus being investigated. A previous study found that POAG was not associated with familial type-I or insulin-dependent diabetes mellitus (Clark 1986). The significant associations found in the Blue Mountains Eye Study were attributed to most patients having type II or non-insulin-dependent diabetes mellitus (Mitchell 1997). To date, no definite evidence for stratified diabetes subtype has yet been found.

CHAPTER VIII

RESULTS, STATISTICAL ANALYSIS & DISCUSSION - MIGRAINE HEADACHE

RESULTS

VIII.I *Familial versus Sporadic Glaucoma Comparison*

Table VIII.1 – Unadjusted odds ratio of migraine in familial glaucoma compared to sporadic glaucoma group.

Odds of Migraine	p-value	odds ratio	95% confidence interval
Unadjusted Odds	0.5	0.88	0.60 – 1.29
Other Risk Factors	0.6	0.90	0.60 - 1.33
GIST scores	0.5	0.87	0.59 – 1.27
Age and Gender	0.5	0.88	0.59 – 1.29

There is no evidence of differences in odds of migraine between familial and sporadic glaucoma groups.

VIII.II *Relationship of odds of having migraine versus GIST score*

The distribution of subjects across GIST scores is summarised in **Table VIII.2.**

Table VIII.2 – Distribution of migraine versus no migraine and odds of migraine as a function of GIST scores in the familial glaucoma, sporadic glaucoma and unaffected groups.

Number		GIST Score						
MIGRAINE	**Group**	**0**	**0.5**	**0.7**	**0.8**	**0.9**	**1**	**Total**
FALSE	Unaffected	26	566					592
	Familial			271	248	164	171	854
	Sporadic			265	190	91	66	612
FALSE Total		**26**	**566**	**536**	**438**	**255**	**237**	**2058**
TRUE	Unaffected	2	148					150
	Familial			74	34	29	23	160
	Sporadic			31	31	5	9	76
TRUE Total		**2**	**148**	**105**	**65**	**34**	**32**	**386**
Grand Total	28	**714**	**641**	**503**	**289**	**269**	**2444**	

There were a greater number of people with no migraine compared to people with migraine across all GIST scores in all three groups. A total of 76 out of 688 (11.0%) in the sporadic glaucoma group had migraine, while a total of 166 out of 1014 (15.8%) in the familial

glaucoma group had migraine (**Table VIII.2**). Overall, a total of 236 glaucoma patients out of 1702 (13.9%) had migraine.

Similar to diabetes mellitus, there were no significant differences among odds across GIST scores 0.7 to 1.0 (Figure VIII).

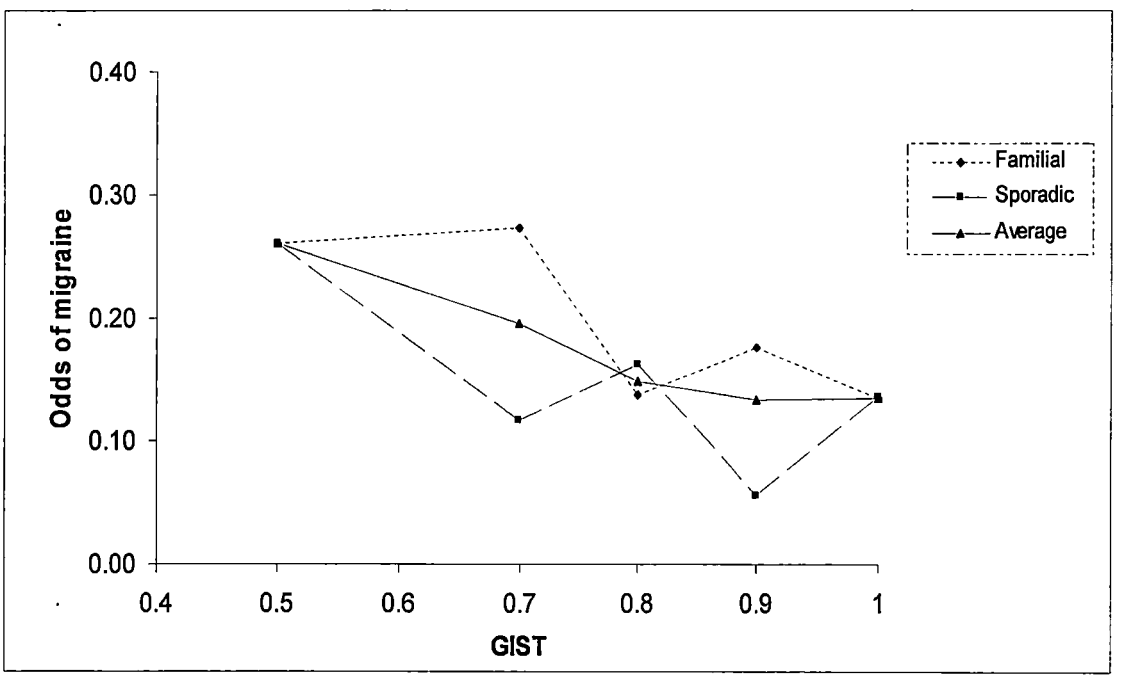


Figure VIII – Comparison of odds of migraine in familial glaucoma, sporadic glaucoma and unaffected groups.

VIII.III *Relationship of Odds of Migraine versus Gender*

Table VIII.3 shows the number of males and females with and without migraine in all three groups.

Table VIII.3 – Distribution of migraine versus no migraine in males and females in familial glaucoma, sporadic glaucoma and unaffected Groups.

Count of Type		Gender		
MIGRAINE	**Type**	**F**	**M**	**Total**
TRUE	Control	111	39	150
	Familial	116	44	160
	Sporadic	55	21	76
TRUE Total		**282**	**104**	**386**
FALSE	Control	329	263	592
	Familial	465	389	854
	Sporadic	333	279	612
FALSE Total		**1127**	**931**	**2058**
Grand Total		**1409**	**1035**	**2444**

In the familial glaucoma group, migraine was found in 116 out of 581 females (20.0%) and in 44 out of 433 males (10.2%). In the sporadic glaucoma group, migraine was found in 55 out of 388 females (14.2%) and in 21 out of 300 males (7.0%). The differences were not statistically significant (OR 1.01; 95%CI 0.55 – 1.90).

VIII.IV *RELATIONSHIP TO DEGREE OF RELATIVES*

Table VIII.4 – Odds of migraine in subgroup of familial glaucoma versus sporadic glaucoma group as a function of degrees of relatives affected with POAG.

Degree of Relative	p-value	odds ratio	95% confidence intervals
1st	0.283	0.798	0.528 – 1.206
2nd	0.664	1.204	0.520 – 2.787
3rd	0.302	1.660	0.635 – 4.342
4th	0.468	0.787	0.413 – 1.501

As shown in **Table VIII.4**, subgroups of degrees of relatives in familial glaucoma demonstrated no significant difference in odds of migraine in comparison to the sporadic glaucoma group.

DISCUSSION

There was a lack of significant association between familial glaucoma and migraine headache in this study (OR 0.88; 95%CI 0.60-1.29). Similarly, no significant association was found in the distribution of migraine between the familial and sporadic glaucoma groups as a function of degree of relatives affected with POAG, GIST scores (OR 0.87; 95%CI 0.59-1.27) or age and gender (OR 0.88; 95%CI 0.59-1.29). Indeed, the association between total POAG and migraine is still under considerable debate.

A preponderance of studies has suggested ocular vasospasm as a risk factor for POAG, particularly low-tension glaucoma (Flammer 1992; Gaspar 1995; Winterkom 1995; Gasser 1989; Drance 1988; Orgul 1994). Migraine, like Raynaud's phenomenon and Prinzmetal variant angina, may be part of a generalised vasospastic tendency (Flammer 1992; Gaspar 1995; Orgul 1994; Gasser 1987; Gasser 1990; Miller 1981; Zahavi 1984). Vasospasm caused by local ocular malformation and vascular dysregulation has also been associated with normal-tension glaucoma (Flammer 1998; Yamamoto 1998).

Calcium channel blockers can potentially inhibit myogenic contraction and hence vasospasm; in selected cases of low-tension glaucoma, treatment with these agents reversed visual field defects (Gaspar 1995; Gasser 1989; Gasser 1987).

In a multivariate study, no difference between low-tension and high-tension glaucoma groups with respect to organic vascular pathologic findings was found (Carter 1990). Despite continuing controversy, it is reasonable to suggest that local perfusion pressure at the nerve head is an important variable in the development of POAG, regardless of IOP (Wilson 1994).

In the population-based Beaver Dam Eye Study, no association between POAG and migraine headache was found (Klein 993). In the more recent Blue Mountains Eye Study, for all age groups combined, there was no significant association between typical migraine headache (16.7% reported history of migraine) and POAG (16.6%) (OR 1.3; 95% CI 0.8-2.2), after multivariate adjustment including glaucoma family history, diabetes, pseudoexfoliation, and hypertension (Wang 1997).

Therefore, there is currently no evidence to suggest an association between familial POAG and migraine headache.

CHAPTER IX

RESULTS, STATISTICAL ANALYSIS & DISCUSSION - CORTICOSTEROIDS

RESULTS

IX.I *Familial versus Sporadic Glaucoma Comparison*

Table IX.1 – Unadjusted odds ratio of corticosteroids use in familial glaucoma compared to sporadic glaucoma group.

Odds of Corticosteroids	p-value	odds ratio	95% confidence interval
Unadjusted Odds	0.75	1.05	0.80 – 1.37
Other Risk Factors	0.68	1.06	0.80 – 1.40
GIST Scores	0.80	1.04	0.80 – 1.37
Age and Gender	0.74	1.05	0.80 – 1.37

There is no evidence of differences in odds of corticosteroid use between familial and sporadic glaucoma groups.

IX.II *Relationship of odds of Corticosteroids use versus GIST score*

The distribution of subjects across GIST scores is summarised in **Table IX.2.**

Table IX.2 – Distribution of corticosteroids use versus no corticosteroids use and odds of corticosteroids use as a function of GIST scores in the familial glaucoma, sporadic glaucoma and unaffected groups.

Number		GIST Score						
STEROIDS	**Group**	**0**	**0.5**	**0.7**	**0.8**	**0.9**	**1**	**Total**
FALSE	Unaffected	25	554					579
	Familial			237	198	133	131	699
	Sporadic			202	145	70	52	469
FALSE Total		**25**	**554**	**439**	**343**	**203**	**183**	**1747**
TRUE	Unaffected	3	160					163
	Familial			108	84	60	63	315
	Sporadic			94	76	26	23	219
TRUE Total		**3**	**160**	**202**	**160**	**86**	**86**	**697**
Grand Total		**28**	**714**	**641**	**503**	**289**	**269**	**2444**

There were a greater number of people who never used corticosteroids compared to people who had used corticosteroids at least once across all GIST scores in all three groups. A total

of 219 out of 688 (31.8%) in the sporadic glaucoma group had ever used corticosteroids at least once, while a total of 315 out of 1014 (31.1%) in the familial glaucoma group had ever used corticosteroids **(Table IX.2)**. Overall, a total of 534 glaucoma patients out of 1702 (31.4%) had ever used corticosteroids.

There are no significant differences among odds across GIST scores 0.7 to 1.0 (Figure IX).

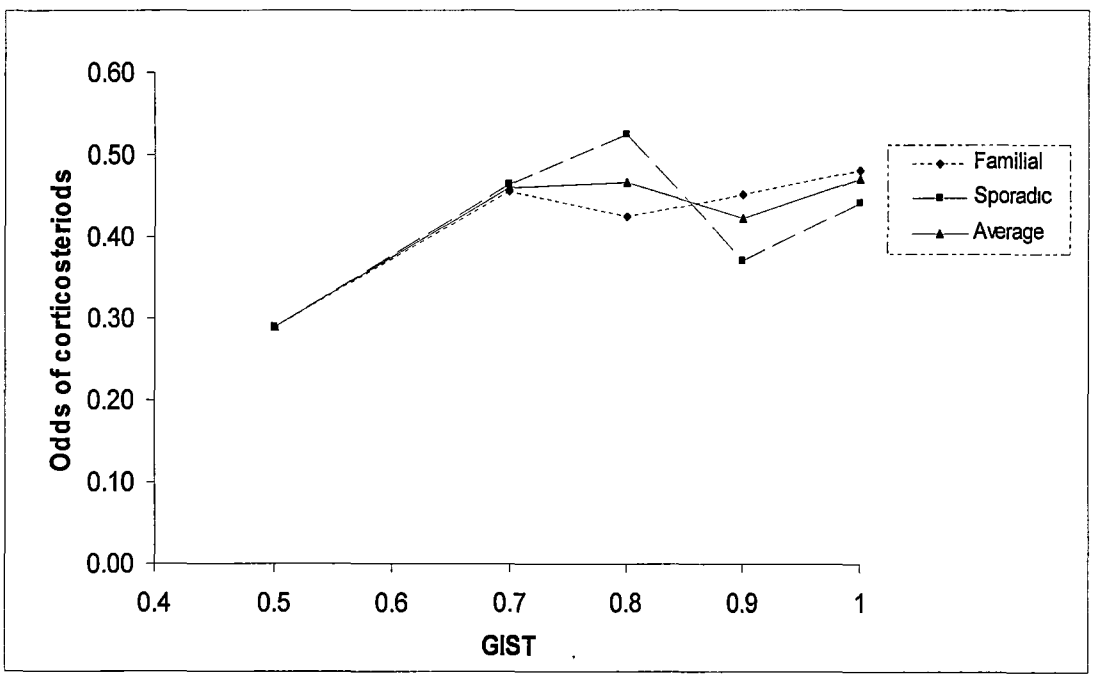


Figure IX – Comparison of odds of corticosteroids in familial glaucoma, sporadic glaucoma and unaffected groups.

IX.III *Relationship of Odds of Corticosteroids use versus Gender*

Table IX.3 shows the number of males and females with and without corticosteroid use in all three groups.

Table IX.3 – Distribution of corticosteroids use versus no corticosteroids use in males and females in familial glaucoma, sporadic glaucoma and unaffected groups.

Count of Type		Gender		
STEROIDS	**Type**	**F**	**M**	**Total**
TRUE	Control	114	49	163
	Familial	183	132	315
	Sporadic	130	89	219
TRUE Total		**427**	**270**	**697**
FALSE	Control	326	253	579
	Familial	398	301	699
	Sporadic	258	211	469
FALSE Total		**982**	**765**	**1747**
Grand Total		**1409**	**1035**	**2444**

In the familial glaucoma group, steroid use was found in 183 out of 581 females (31.5%) and in 132 out of 433 males (30.5%). In the sporadic glaucoma group, steroid use was found in 130 out of 388 females (33.5%) and in 89 out of 300 males (29.7%). The differences are not statistically significant (OR 0.95; 95%CI 0.67 – 1.40).

IX.IV *Relationship to Degree of Relatives*

Table IX.4 – Odds of corticosteroid use in subgroups of familial glaucoma versus sporadic glaucoma group as a function of degrees of relative affected with POAG.

Degree of Relative	p-value	odds ratio	95% confidence intervals
1st	0.393	1.139	0.846 – 1.533
2nd	0.992	0.997	0.582 – 1.710
3rd	0.999	1.000	0.578 – 1.732
4th	0.300	0.785	0 496 – 1 242

As shown in **Table IX.4**, subgroups of degrees of relatives in familial glaucoma demonstrated no significant difference in odds of corticosteroid use compared to the sporadic glaucoma group.

DISCUSSION

In this study, ever use of corticosteroids was not significantly different in terms of prevalence in the familial POAG group compared to the sporadic glaucoma group (OR 1.05; 95%CI 0.80-1.37). Conversely, no significant association was found in the distribution of corticosteroid use between the familial and sporadic glaucoma groups as a function of degree of relatives, GIST scores (OR 1.04; 95%CI 0.80-1.37) or age and gender (OR 1.05; 95%CI 0.80-1.37). As this is a cross-section study, it lacks power to show temporal relationship between the two variables and provides no information on dose-effect response. Indeed, any true relationship between POAG and steroid responsiveness would be better explored in a smaller subgroup of patients whose steroid responsiveness is known, or can be tested by challenge with topical steroids.

However, many studies have demonstrated that the use of oral or topical ophthalmic corticosteroids in a minority of people, called 'steroid responders', can lead to varying degrees of elevated IOP and predispose them to developing POAG (Becker 1963; 1965; Williamson 1969; Mitchell 1999).

An earlier study reported that nearly all persons with a diagnosis of POAG were moderate-to-high responders (Becker 1964). Ninety percent of patients with POAG were high responders to a six-week course of topical ocular betamethasone, during which their IOPs showed marked elevation (>30mmHg). The remaining 10% of patients with POAG showed

moderate response with a moderate elevation of IOP (22-30mmHg). Steroid responders in the normal population were also shown to have a higher risk of developing POAG over time (Lewis 1988; Kitazawa 1981).

The Blue Mountains Eye Study reported a strong association between ever use of inhaled corticosteroids and findings of glaucoma or OH in persons with a family history of glaucoma (OR 2.6; CI 1.2-5.8). Moreover, the risk increased with higher doses (OR 6.3; CI 1.0-38.6) for persons who used more than four puffs per day. However, no statistically significant association was found for persons with any family history of glaucoma, adding further support for a genetic mechanism in the association.

A genetic basis for steroid-induced OH was postulated by some investigators in the 1960s (Becker 1964; Armaly 1967), but disputed by others (Francois 1966). Clinical studies have demonstrated that inheritance of steroid response may have an autosomal recessive pattern and may be associated with inheritance of glaucoma (Davies 1968; Bartlett 1993) in a rather complex manner (Clark 1995). It is interesting that both siblings and offspring of patients with POAG have increased responsiveness to steroids (30 and 25% respectively are high responders) (Richardson 1997). Recently, the discovery of the trabecular meshwork-induced glucocorticoid response (TIGR) gene or MYOC at the GLC1A locus and its multiple disease-causing mutations in some 5% of hereditary glaucoma has added further support for the genetic basis (Fingert 1999).

One of its proteins, originally called TIGR, showed time-dependent induction with dexamethasone treatment over several weeks (Polansky 1997). Polansky and collaborators (1997) have also reported repression of steroid induction of MYOC mRNA in human trabecular meshwork *in vitro* by addition of the nonsteroidal anti-inflammatory drug diclofenac. Whether pharmacologic manipulation of the expression of this gene will benefit a subset of patients with POAG is yet to be determined. The promoter region of MYOC has been identified and contains regions (consensus motifs) that are responsive to glucocorticoids and a number of other hormones (Polansky 1998). This led to the hypothesis that sequence changes here or in other regulatory regions of MYOC or other glaucoma genes (Alward 1998; Kubota 1998) could be involved in development of steroid-induced glaucoma. Recently, however, a study using a cynomologus monkey model and DNA-mutation screening of GLC1Amethods did not support the hypothesis and found no statistically significant evidence for a link between MYOC mutations and steroid-induced OH (Fingert 2001). Furthermore, in a Korean study, MYOC did not seem to be related to steroid-induced glaucoma (Kee 1997).

At present, there is no significant difference in the odds of ever use of corticosteroids between familial and sporadic glaucoma groups.

CHAPTER X

RESULTS, STATISTICAL ANALYSIS & DISCUSSION - SMOKING

"When you know a thing, to hold that you know it, and when you do not know it, to admit that you do not know it – this is true knowledge"

(Confucius)

RESULTS

X.I *Familial versus Sporadic Glaucoma Comparison*

Table X.1 – Unadjusted odds ratio smoking in familial glaucoma compared to sporadic glaucoma group.

Odds of Smoking	p-value	odds ratio	95% confidence interval
Unadjusted Odds	0.19	0.75	0.49 – 1.15
Other Risk factors	0.19	0.75	0.49 – 1.15
GIST scores	0.17	0.74	0.48 – 1.14
Age and Gender	0.20	0.75	0.49 – 1.16

There is no evidence of differences in odds of smoking between familial and sporadic glaucoma groups.

X.II *Relationship of odds of Smoking versus GIST score*

The distribution of subjects across GIST scores is summarized in **table X.2** below.

Table X.2 – Distribution of smoking versus no smoking and odds of smoking as a function of GIST scores in the familial glaucoma, sporadic glaucoma and unaffected groups.

Number		GIST Score						
SMOKING	**Group**	**0**	**0.5**	**0.7**	**0.8**	**0.9**	**1**	**Total**
FALSE	Unaffected	28	582					610
	Familial			295	250	170	174	889
	Sporadic			264	199	91	68	622
FALSE Total	**28**	**582**	**559**	**449**	**261**	**242**	**2121**	
TRUE	Unaffected		132					132
	Familial			50	32	23	20	125
	Sporadic			32	22	5	7	66
TRUE Total		**0**	**132**	**82**	**54**	**28**	**27**	**323**
Grand Total		**28**	**714**	**641**	**503**	**289**	**269**	**2444**

There was a greater number of people who never smoked compared to people who ever smoked across all GIST scores in all three groups. A total of 66 out of 686 (9.6%) in the

sporadic glaucoma group had smoked, while a total of 125 out of 1014 (12.3%) in the familial glaucoma group had smoked (**Table X.2**). Overall, a total of 191 glaucoma patients out of 1702 (11.2%) had smoked.

There were no significant differences among odds of smoking across GIST scores 0.7 to 1.0 (Figure X).

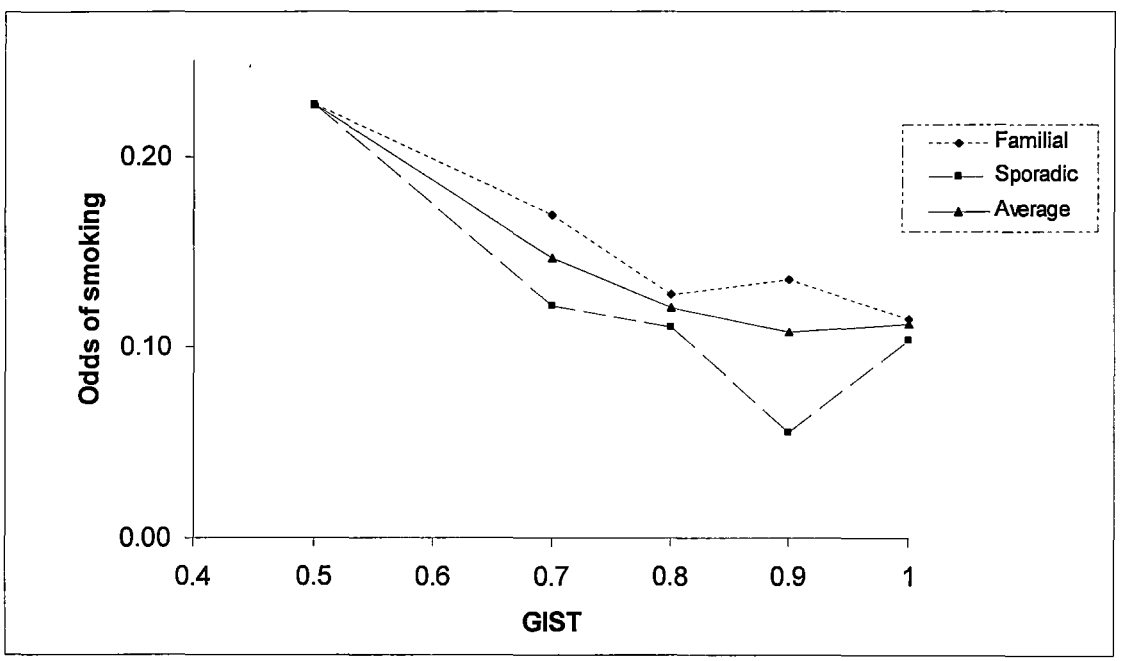


Figure X – Comparison of odds of smoking in familial glaucoma, sporadic glaucoma and unaffected groups.

X.III *Relationship of Odds of Smoking versus Gender*

Table X.3 shows the number of males and females with and without smoking in all three groups.

Table X.3 – Distribution of smoking versus no smoking in males and females in familial glaucoma, sporadic glaucoma and unaffected groups.

Count of Type		Gender		
SMOKING	**Type**	**F**	**M**	**Total**
TRUE	Control	66	66	132
	Familial	70	55	125
	Sporadic	24	42	66
TRUE Total		**16**	**163**	**323**
FALSE	Control	374	236	610
	Familial	511	378	889
	Sporadic	364	258	622
FALSE Total		**1249**	**872**	**2121**
Grand Total		**1409**	**1035**	**2444**

In the familial glaucoma group, 70 out of 581 females (12.0%) and 55 out of 433 males (12.7%) had smoked. In the sporadic glaucoma group, 24 out of 388 females (6.2%) and 42 out of 300 males (14.0%) had ever smoked. The differences were not statistically significant (OR 2.23; 95%CI 1.0 – 4.1).

IX.IV *RELATIONSHIP TO DEGREE OF RELATIVE*

Table X.4 – Odds of smoking in subgroups of familial glaucoma versus sporadic glaucoma group as a function of degrees of relatives affected with POAG.

Degree of Relative	p-value	odds ratio	95% confidence intervals
1st	0.379	0.810	0.508 – 1.294
2nd	0.169	0.589	0.277 – 1.253
3rd	0.692	0.841	0.357 – 1.982
4th	0.181	0.628	0.318 – 1.242

As shown in **Table X.4,** subgroups of degrees of relative in familial glaucoma demonstrated no significant difference in odds of having ever smoked in comparison to the sporadic glaucoma group.

DISCUSSION

Glaucoma has been reported to be associated with increased alcohol consumption (Kahn 1980) and cigarette smoking (Wilson 1987). However, in the Blue Mountains Eye study, smoking was demonstrated not to be statistically significantly associated with POAG, and adding smoking (current versus non-current or ever versus never) into the multivariate model comparing migraine and POAG did not alter results (Wang 1997).

In this study, smoking was not significantly related to familial POAG (OR 0.75; 95%CI 0.49-1.15). Similarly, no significant association was found in the distribution of smoking between the familial and sporadic glaucoma groups as a function of degree of relatives, GIST scores (OR 0.74; CI 0.48-1.14) or age and gender (OR 0.75; CI 0.49-1.16).

CHAPTER XI

RESULTS, STATISTICAL ANALYSIS & DISCUSSION - ATHEROSCLEROSIS

RESULTS

XI.I *Familial versus Sporadic Glaucoma Comparison*

Table XI.1 – Unadjusted and Adjusted Odds ratios and confidence intervals of atherosclerosis in familial glaucoma compared to sporadic glaucoma group.

Adjustment	p-value	odds ratio	95% confidence interval
None	0.03	0.70	0.50 – 0 97
Other Risk factors	0.04	0.71	0.51 – 0.99
GIST Scores	0.07	0.74	0.53 – 1.03
Age and Gender	0.03	0.69	0.49 - 0.95

The unadjusted odds of atherosclerosis was significantly different in the familial glaucoma group compared to the sporadic glaucoma group (OR 0.70; 95%CI 0.50-0.97). However, after adjusting for other clinical risk factors, the relationship is weakened (OR 0.71; 95%CI 0.51-0.99).

XI.II *Relationship of odds of having Atherosclerosis versus GIST score*

The distribution of subjects across GIST scores is summarised in **Table XI.2** below.

Table XI.2 – Distribution of atherosclerosis versus no atherosclerosis and odds of atherosclerosis as a function of GIST scores in the familial glaucoma, sporadic glaucoma and unaffected groups.

Number		GIST Score						
ATHEROSCLE.	Group	0	0.5	0.7	0.8	0.9	1	Total
TRUE	Unaffected	6	65					71
	Familial			72	47	45	54	218
	Sporadic			65	35	16	13	129
TRUE Total		**6**	**65**	**137**	**82**	**61**	**67**	**418**
FALSE	Unaffected	22	649					671
	Familial			273	235	148	140	796
	Sporadic			231	186	80	62	559
FALSE Total		**22**	**649**	**504**	**421**	**228**	**202**	**2026**
Grand Total		**28**	**714**	**641**	**503**	**289**	**269**	**2444**

There were a greater number of people who had no atherosclerosis compared to people who had atherosclerosis across all GIST scores in all 3 groups. A total of 129 out of 688 (18.8%) in the sporadic glaucoma group had atherosclerosis, while a total of 218 out of 1014 (21.5%)

in the familial glaucoma group had athersclerosis (**Table XI.2**). Overall, a total of 347 glaucoma patients out of 1702 (20.4%) had atherosclerosis.

There were no significant differences among odds of atherosclerosis across GIST scores 0.7 to 1.0 (Figure XI).

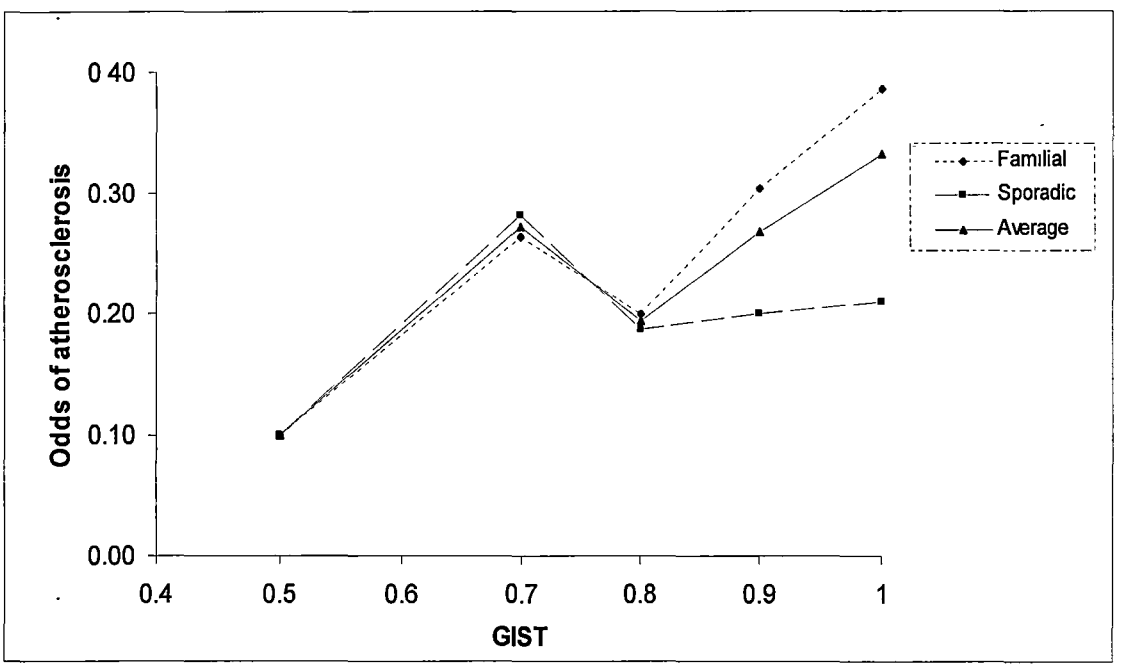


Figure XI – Comparison of odds of atherosclerosis in familial glaucoma, sporadic glaucoma and unaffected groups.

It was interesting to note the difference between the familial glaucoma and sporadic group in respect to diminished likelihood of atherosclerosis. It became non-significant when the difference in GIST levels between the groups was removed (OR 0.74; 95%CI 0.53-1.03).

XI.III Relationship of Odds of Atherosclerosis versus Gender

Table XI.3 shows the number of males and females with and without atherosclerosis in all three groups.

Table XI.3 – Distribution of atherosclerosis versus no atherosclerosis in males and females in familial glaucoma, sporadic glaucoma and unaffected Groups.

Count of Type		Gender		
ATHEROSC	**Type**	**F**	**M**	**Total**
TRUE	Control	42	29	71
	Familial	114	104	218
	Sporadic	70	59	129
TRUE Total		**226**	**192**	**418**
FALSE	Control	398	273	671
	Familial	467	329	796
	Sporadic	318	241	559
FALSE Total		**1183**	**843**	**2026**
Grand Total		**1409**	**1035**	**2444**

In the familial glaucoma group, atherosclerosis was found in 114 out of 581 females (19.6%) and in 104 out of 433 males (24.0%). In the sporadic glaucoma group, atherosclerosis was found in 70 out of 388 females (18.0%) and in 59 out of 300 males (19.7%). These differences were not statistically significant (OR 1.09; 95%CI 0.70 – 1.70). Indeed, the odds ratio of atherosclerosis in familial glaucoma compared to sporadic glaucoma is not affected

by adjustments for age and gender (OR 0.69; 95%CI 0.49-0.95), which is not surprising given that the two groups have a similar age and gender profile.

XI.IV *RELATIONSHIP TO DEGREE OF RELATIVE*

Table XI.4 – Odds of atherosclerosis in subgroups of familial glaucoma versus sporadic glaucoma group as a function of degrees of relatives affected with POAG.

Degree of Relative	p-value	odds ratio	95% confidence intervals
1st	0.105	0.745	0.523 – 1.063
2nd	0.351	0.741	0.395 – 1.390
3rd	0.053	0.553	0.303 – 1.008
4th	0.068	0.610	0.358 – 1.038

Similar to GIST scores, no significant association was found in the distribution of atherosclerosis between the familial and sporadic glaucoma groups as a function of degree of relatives in the familial group.

It seems that the analysis of the distribution of atherosclerosis across degree of relative in the familial glaucoma group does not support an atherosclerosis-familial POAG relationship. If a genetic effect exists, it is likely to be stronger in the first-degree relatives compared to the more distant blood relatives. Despite being limited by the relatively wide confidence intervals in this study, the odds of atherosclerosis compared to sporadic glaucoma in 1st degree

relatives of familial glaucoma is 0.745 (95%CI 0.523-1.063) versus 0.553 (95%CI 0.303-1.008) and 0.610 (0.358-1.038) in 3rd and 4th degrees of relatives respectively. This suggests that a genetic association between atherosclerosis and familial POAG is unlikely.

DISCUSSION

The odds of atherosclerosis appear to be weakly significantly different between the familial glaucoma and sporadic glaucoma groups (OR 0.70; 95%CI 0.50-0.97) (p=0.03), and is unaffected by adjustments for other risk factors (OR 0.71; 95%CI 0.51-0.99) or age and gender (OR 0.69; 95%CI 0.49-0.95). However, standardising GIST scores rendered the association non-significant (OR 0.74; 95%CI 0.53-1.03). Furthermore, there is no significant association between degrees of relatives in the familial glaucoma group and sporadic glaucoma. With relatively large confidence intervals, these findings are likely to reflect a chance finding, but an independent study can provide more definite answers.

Indeed, the degree of atherosclerosis may not correlate closely with self-reported end-organ symptoms, and any true relationship between POAG and atherosclerosis might be better explored by correlating with the actual extent of disease measured with carotid ultrasonography.

At present, therefore, there is insufficient evidence to show a significant association between atherosclerosis and familial POAG.

CHAPTER XII

RESULTS, STATISTICAL ANALYSIS & DISCUSSION – COLD EXTREMITIES

RESULTS

XII.I *Familial versus Sporadic Glaucoma Comparison*

Table XII.1 – Unadjusted odds ratio of cold extremities in familial glaucoma compared to sporadic glaucoma group.

Odds of Cold Extremities	p-value	odds ratio	95% confidence interval
Unadjusted Odds	0.5	0.92	0.71 – 1.20
Other Risk factors	0.8	0.97	0.73 – 1.27
GIST scores	0.6	0.93	0.71 – 1.21
Age and Gender	0.5	0.92	0.70 – 1.19

There is no evidence of differences in odds of cold extremities between familial and sporadic glaucoma groups.

XII.II *Relationship of odds of having Cold Extremities versus GIST score*

The distribution of subjects across GIST scores is summarised in **Table XII.2** below.

Table XII.2 – Distribution of cold extremities versus no cold extremities and odds of cold extremities as a function of GIST scores in the familial glaucoma, sporadic glaucoma and unaffected groups.

Number		GIST Score						
COLD EXTREM.	**Group**	**0**	**0.5**	**0.7**	**0.8**	**0.9**	**1**	**Total**
FALSE	Unaffected	25	552					577
	Familial			224	192	117	126	659
	Sporadic			205	145	66	51	467
FALSE Total		**25**	**552**	**429**	**337**	**183**	**177**	**1703**
TRUE	Unaffected	3	162					165
	Familial			121	90	76	68	355
	Sporadic			91	76	30	24	221
TRUE Total		**3**	**162**	**212**	**166**	**106**	**92**	**741**
Grand Total		**28**	**714**	**641**	**503**	**289**	**269**	**2444**

There were a greater number of people who had no cold extremities compared to people who had cold extremities across all GIST scores in all three groups. A total of 221 out of 688 (32.1%) in the sporadic glaucoma group had cold extremities, while a total of 355 out of

1014 (35.1%) in the familial glaucoma group had cold extremities (**Table XII.2**). Overall, a total of 576 glaucoma patients out of 1702 (33.8%) had cold extremities.

There were no significant differences among odds of cold extremities across GIST scores 0.7 to 1.0 (Figure XII).

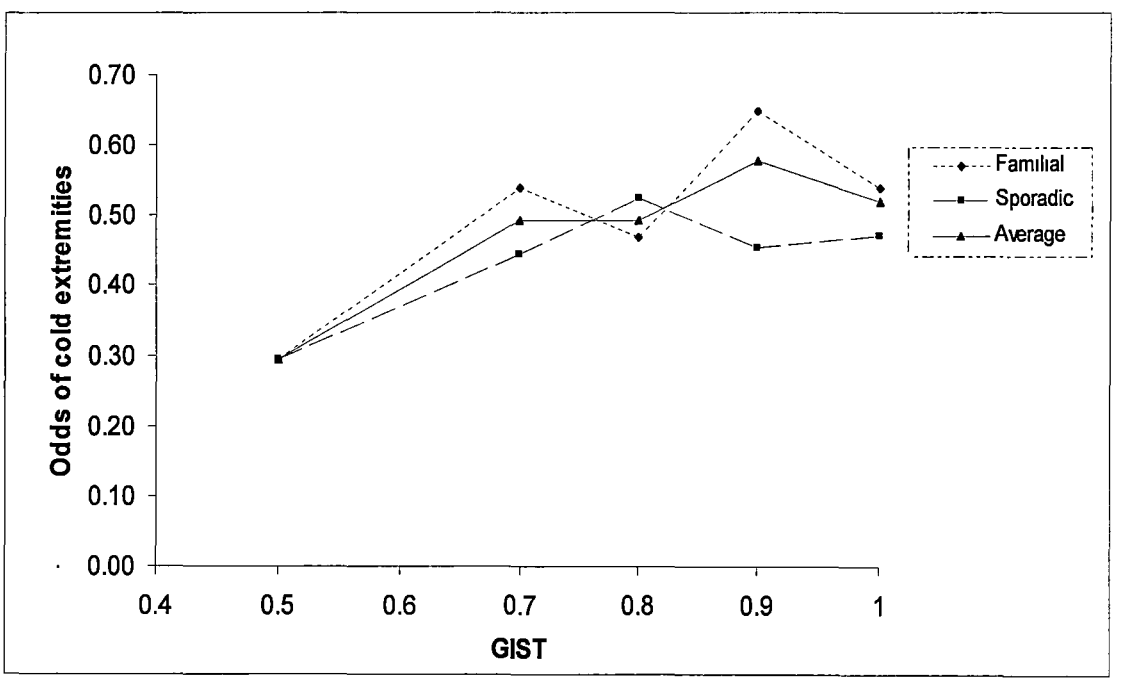


Figure XII – Comparison of odds of cold extremities in familial glaucoma, sporadic glaucoma and unaffected groups.

XII.III *Relationship of Odds of Cold Extremities versus Gender*

Table XII.3 shows the number of males and females with and without cold extremities in all three groups

.

Table XII.3 – Distribution of cold extremities versus no cold extremities in males and females in familial glaucoma, sporadic glaucoma and unaffected groups.

Count of Type		Gender		
COLD EXTREM	**Type**	**F**	**M**	**Total**
TRUE	Control	124	41	165
	Familial	227	128	355
	Sporadic	125	96	221
TRUE Total		**476**	**265**	**741**
FALSE	Control	316	261	577
	Familial	354	305	659
	Sporadic	263	204	467
FALSE Total		**933**	**770**	**1703**
Grand Total		**1409**	**1035**	**2444**

In the familial glaucoma group, cold extremities were found in 227 out of 581 females (39.1%) and in 128 out of 433 males (29.6%). In the sporadic glaucoma group, cold extremities were found in 125 out of 388 females (32.2%) and in 96 out of 300 males (32.0%). The differences were not statistically significant (OR 0.73; 95%CI 0.52 – 1.04).

XII.IV *Relationship to Degree of Relative*

Table XII.4 – Odds of cold extremities in subgroups of familial glaucoma versus sporadic glaucoma group as a function of degrees of relative affected with POAG.

Degree of Relative	p-value	odds ratio	95% confidence intervals
1st	0.451	0.895	0.671 – 1.194
2nd	0.506	1.205	0.695 – 2.089
3rd	0.835	1.060	0.613 – 1.834
4th	0.329	0.797	0.505 – 1.257

As shown in **Table XII.4**, subgroups of degrees of relative in familial glaucoma demonstrated no significant difference in odds of cold extremities compared to the sporadic glaucoma group.

DISCUSSION

There was no significant association between cold extremities, which reflect vasospasm of peripheral arteries, and familial POAG (OR 0.92; 95%CI 0.71-1.20). Conversely, no significant association was found in the distribution of cold extremities between the familial and sporadic glaucoma groups as a function of degree of relatives, GIST scores (OR 0.93; 95%CI 0.71-1.21) or age and gender (OR 0.92; 95%CI 0.7-1.19).

The lack of a significant association between cold extremities and familial POAG supports the absence of a significant association between migraine and familial POAG, which presumably occurs via a similar vasospastic mechanism.

CHAPTER XIII

RESULTS, STATISTICAL ANALYSIS & DISCUSSION – BLOOD TRANSFUSION

RESULTS

XIII.I *Familial versus Sporadic Glaucoma Comparison*

Table XIII.1 – Unadjusted odds ratio of blood transfusion in familial glaucoma compared to sporadic glaucoma group.

Odds of Transfusion	p-value	odds ratio	95% confidence interval
Unadjusted Odds	0.96	0.99	0.73 – 1.36
Other Risk factors	0.77	1.05	0.76 – 1.45
GIST scores	0.99	0.99	0.73 – 1.37
Age and Gender	0.91	0.98	0.72 – 1.70

There is no evidence of differences in odds of blood transfusion between familial and sporadic glaucoma groups.

XIII.II Relationship of odds of having Transfusion versus GIST score

The distribution of subjects across GIST scores is summarised in **Table XIII.2** below.

Table XIII.2 – Distribution of transfusion versus no transfusion and odds of transfusion as a function of GIST scores in the familial glaucoma, sporadic glaucoma and unaffected groups.

Number		GIST Score						
TRANSFUSION	**Group**	**0**	**0.5**	**0.7**	**0.8**	**0.9**	**1**	**Total**
FALSE	Unaffected	25	617					642
	Familial			269	230	142	131	804
	Sporadic			223	173	78	63	537
FALSE Total		**25**	**617**	**492**	**403**	**233**	**213**	**1983**
TRUE	Unaffected	3	97					100
	Familial			76	52	38	44	210
	Sporadic			73	48	18	12	151
TRUE Total		**3**	**97**	**149**	**100**	**56**	**56**	**461**
Grand Total		**28**	**714**	**641**	**503**	**289**	**269**	**2444**

There were a greater number of people who had had no blood transfusion compared to people who had had transfusion across all GIST scores in all three groups. A total of 151 out of 688 (21.9%) in the sporadic glaucoma group had had a transfusion, while a total of 210

out of 1014 (20.7%) in the familial glaucoma group had a had transfusion (**Table XIII.2**). Overall, a total of 361 glaucoma patients out of 1702 (21.2%) had had a transfusion.

There was no significant differences among odds of blood transfusion across GIST scores 0.7 to 1.0 (Figure XIII).

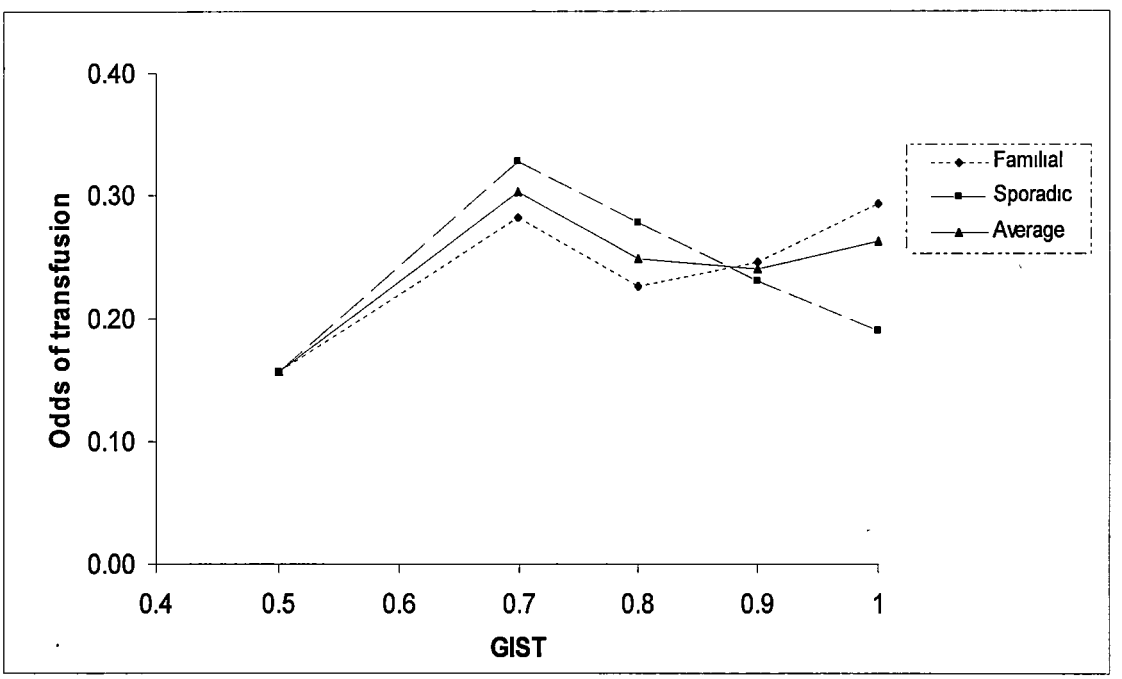


Figure XIII – Comparison of odds of transfusion in familial glaucoma, sporadic glaucoma and unaffected groups.

XIII.III *Relationship of Odds of Transfusion versus Gender*

Table XIII.3 shows the number of males and females with and without transfusion in all three groups.

Table XIII.3 – Distribution of transfusion versus no transfusion in males and females in familial glaucoma, sporadic glaucoma and unaffected Groups.

Count of Type		**Gender**		
TRANSFUSION	**Type**	**F**	**M**	**Total**
TRUE	Control	71	29	100
	Familial	131	79	210
	Sporadic	103	48	151
TRUE Total		**305**	**156**	**461**
FALSE	Control	369	273	642
	Familial	450	354	804
	Sporadic	285	252	537
FALSE Total		**1104**	**879**	**1983**
Grand Total		**1409**	**1035**	**2444**

In the familial glaucoma group, 131 out of 581 females (22.5%) and 79 out of 433 males (18.2%) had had a blood transfusion. In the sporadic glaucoma group, 103 out of 388 females (26.5%) and 48 out of 300 males (16.0%) had had a blood transfusion. The differences were not statistically significant (OR 0.77; 95%CI 0.50 – 1.21).

XIII.IV *Relationship to Degree of Relative*

Table XIII.4 – Odds of blood transfusion in subgroups of familial glaucoma versus sporadic glaucoma group as a function of degree of relative affected with POAG.

Degree of Relative	p-value	odds ratio	95% confidence intervals
1st	0.909	1.020	0.723 – 1.439
2nd	0.045	0.565	0.323 – 0.988
3rd	0.839	1.070	0.557 – 2 055
4th	0.283	1.391	0.761 – 2.541

As shown in **Table XIII.4**, subgroups of degrees of relative in the familial glaucoma group demonstrated no significant difference in odds of blood transfusion compared tothe sporadic glaucoma group, except for the 2nd degree relatives (p=0.045). However, it would appear to be reasonable to attribute this difference to chance variation since in the absence of any difference, 1 in 20 will on average have a p-value less than 0.5

DISCUSSION

There was no significant association between blood transfusion, which presumably put haemodynamic stress on blood vessels, and familial POAG (OR 0.99; 95%CI 0.73-1.36). Conversely, no significant association was found in the distribution of blood transfusion between the familial and sporadic glaucoma groups as a function of degree of relatives, GIST scores (OR 0.99; 95%CI 0.73-1.37) or age and gender (OR 0.98; 95%CI 0.72-1.70).

CHAPTER XIV

RESULTS, STATISTICAL ANALYSIS & DISCUSSION - THYROID DISORDERS

RESULTS

XIV.I *Familial versus Sporadic Glaucoma Comparison*

Table XIV.1 – Unadjusted odds ratio of thyroid disorder in familial glaucoma compared to sporadic glaucoma group.

Odds of Thyroid Disorder	p-value	odds ratio	95% confidence interval
Unadjusted Odds	0.7	0.92	0.61 – 1.38
Other Risk factors	0.8	0.94	0.62 – 1.44
GIST scores	0.6	0.88	0.58 – 1.34
Age and Gender	0.6	0.90	0.59 – 1.36

There is no evidence of differences in odds of thyroid disorder between familial and sporadic glaucoma groups.

XIV.II *Relationship of odds of Thyroid disorders versus GIST score*

The distribution of subjects across GIST scores is summarised in **Table XIV.2** below.

Table XIV.2 – Distribution of thyroid disorders versus no thyroid disorders and odds of thyroid disorders as a function of GIST scores in the familial glaucoma, sporadic glaucoma and unaffected groups.

Number		GIST Score						
THYRIOD	**Group**	**0**	**0.5**	**0.7**	**0.8**	**0.9**	**1**	**Total**
FALSE	**Unaffected**	23	657					680
	Familial			295	248	171	178	892
	Sporadic			262	196	91	66	615
FALSE Total		**23**	**657**	**557**	**444**	**262**	**244**	**2187**
TRUE	**Unaffected**	5	57					62
	Familial			50	34	22	16	122
	Sporadic			34	25	5	9	73
TRUE Total		**5**	**57**	**84**	**59**	**27**	**25**	**257**
Grand Total		**28**	**714**	**641**	**503**	**289**	**269**	**2444**

There were a greater number of people who had no thyroid disorders (n=2187) compared to people who had thyroid disorders (n=257) across all GIST scores in all three groups. A total

of 73 out of 688 (10.6%) in the sporadic glaucoma group had thyroid disorders, while a total of 122 out of 1014 (12.0%) in the familial glaucoma group had thyroid disorders (**Table XIV.2**). Overall, a total of 195 glaucoma patients out of 1702 (11.5%) had thyroid disorders.

There were no significant differences among odds of thyroid disorder across GIST scores 0.7 to 1.0 (Figure XIV).

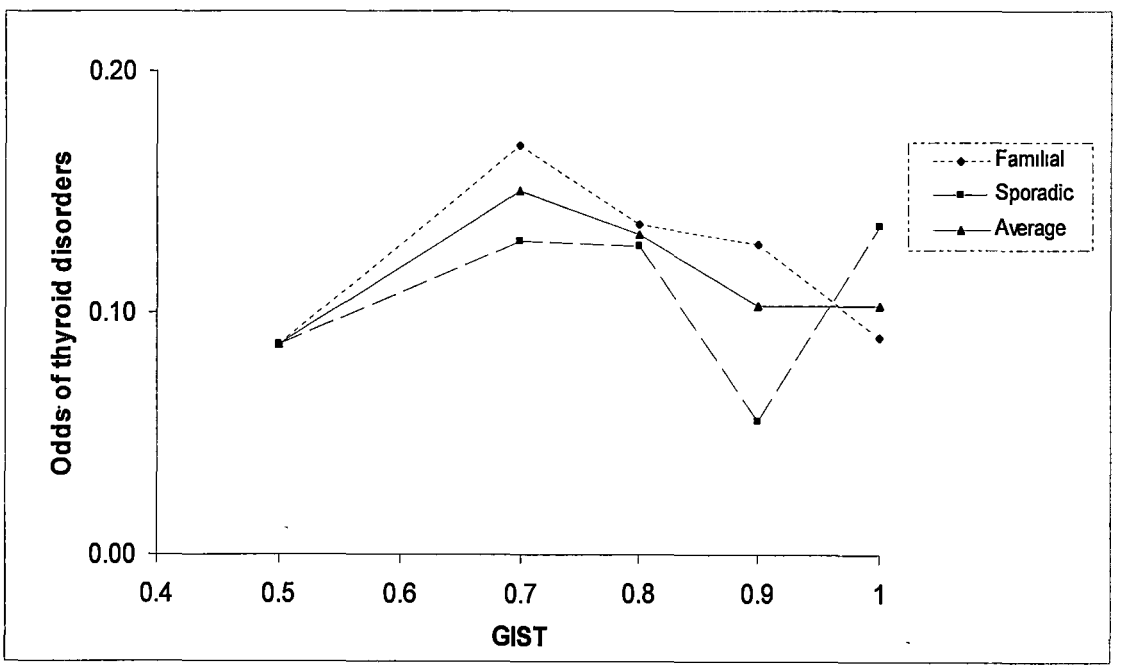


Figure XIV – Comparison of odds of thyroid disorders in familial glaucoma, sporadic glaucoma and unaffected groups.

XIV.III *Relationship of Odds of Thyroid Disorders versus Gender*

Table XIV.3 shows the number of males and females with and without thyroid disorders in all 3 groups.

Table XIV.3 – Distribution of thyroid disorders versus no thyroid disorders in males and females in familial glaucoma, sporadic glaucoma and unaffected Groups.

Count of Type		Gender		
THYROID	**Type**	**F**	**M**	**Total**
TRUE	Control	54	8	62
	Familial	102	20	122
	Sporadic	57	16	73
TRUE Total		**213**	**44**	**257**
FALSE	Control	386	294	680
	Familial	479	413	892
	Sporadic	331	284	615
FALSE Total		**1196**	**991**	**2187**
Grand Total		**1409**	**1035**	**2444**

In the familial glaucoma group, thyroid disorders were found in 102 out of 581 females (17.6%) and in 20 out of 433 males (4.6%). In the sporadic glaucoma group, thyroid

disorders were found in 57 out of 388 females (14.7%) and in 16 out of 300 males (5.3%). The differences were not statistically significant (OR 0.70; 95% CI 0.34 – 1.50).

XIV.IV *Relationship to Degree of Relative*

Table XIV.4 – Odds of thyroid disorders in subgroups of familial glaucoma versus sporadic glaucoma group as a function of degrees of relative affected with POAG.

Degree of Relative	p-value	odds ratio	95% confidence intervals
1st	0.762	1.074	0 676 – 1.709
2nd	0.953	1.026	0.440 – 2 393
3rd	0.015	0.434	0.221 – 0.851
4th	0.733	0.884	0.435 – 1.795

As shown in **Table XIV.4**, there was a significant difference in odds of thyroid disorder between familial glaucoma subjects with an affected third- or fourth-degree relative compared to sporadic glaucoma subjects. However the association became non-significant when familial glaucoma subjects with an affected 1st, 2nd or 4th degree relative was considered. This is likely to be a chance variation given 1 in 60 will produce a similar difference and a dilutional effect of a genetic association is not demonstrated.

DISCUSSION

At present, there is insufficient evidence to support a significant association between thyroid disorders and familial POAG (OR 0.92; 95%CI 0.61-1.38). Similarly, no significant association was found in the distribution of thyroid disorders between the familial and sporadic glaucoma groups as a function of degree of relatives, GIST scores (OR 0.88; 95%CI 0.58-1.34) or age and gender (OR 0.90; 95%CI 0.59-1.36).

CHAPTER XV

RESULTS, STATISTICAL ANALYSIS & DISCUSSION – RELATIONSHIP OF RISK FACTORS TO 'CONTROLS'

XV.I *Comparing 'Controls' versus Familial and Sporadic Glaucoma groups*

Table XV.1 summarises the odds of various clinical risk factors in the total (familial and sporadic) glaucoma group compared to the 'control' unaffected group.

Risk Factor	p-value	Odds Ratio	95% Confidence Intervals
Hypertension	0.20	1.162	0.92 – 1.50
Smoking	0.99	1.00	0.75 – 1.30
Diabetes	<0.01	2.67	1.70 – 4 30
Transfusion	0.79	1.04	0.79 – 1.40
Atherosclerosis	0.89	0.98	0.72 – 1.30
Cold extremities	0.05	1.27	1.01 – 1.60
Thyroid disorder	0.67	0.93	0.66 – 1.30
Migraine	0 22	0.85	0.65 – 1.10
Steriods	<0 01	1 42	1.10 – 1.80

XV.1 – Age-Gender adjusted Odds Ratios and Confidence Intervals for various clinical risk factors comparing total glaucoma and 'control' unaffected group.

As shown in table XV.1, the prevalence of diabetes mellitus is significantly higher in the total glaucoma group compared to the 'control' unaffected group (age-gender adjusted OR 2.67; 95%CI 1.7-4.3). Similarly, ever use of corticosteroids is significantly more prevalent in the total glaucoma group in comparison to the 'control' group (age-gender adjusted OR 1.42; 95%CI 1.1-1.8). No significant difference is found for the other seven clinical risk factors (hypertension, smoking, transfusion, atherosclerosis, cold extremities, thyroid disorders and migraine).

DISCUSSION

In this study, diabetes mellitus is significantly more prevalent in the total glaucoma group compared to the 'control' group (age-gender adjusted OR 2.67; 95%CI 1.7-4.3) in a magnitude similar to that found by the Blue Mountains Eye Mountain (age-gender adjusted OR 2.82; 95%CI 1.35-5.87).

Ever use of corticosteroids is also significantly associated with the total glaucoma group compared to the 'control' group (age-gender adjusted OR 1.42; 95%CI 1.1-1.8).

For the other seven risk factors there is no evidence to reject the hypothesis that odds of true:false is the same for the total glaucoma and 'control' groups. However, as discussed in chapter V, the unaffected 'control' group has a substantially younger age profile (median age52 years) than the total glaucoma group (median age 74 years) and this may account for the

differences. It must be noted that age-adjusted comparisons are sensible given that the difference in the age patterns is a characteristic of the groups rather than an artefact of the sampling process. Since the risk of glaucoma is age-dependent, to make an age adjustment may create an artificial population unlike that in the natural world. That is, the assumption that prevalence with age increases in the same manner for the total glaucoma and 'control' groups may not be valid.

For gender, however, there is no significant difference in the male/female distribution in the total glaucoma group compared to the 'control' group (OR 1.102; 95% CI 0.925 – 1.313).

Comparisons between the glaucoma groups and the unaffected 'control' group must be considered carefully. As the 'control' group consists of individuals who have a GIST score of 0.5 or less, and may be related to a glaucoma sufferer, they may not truly represent the normal population with no definite glaucoma. Indeed, some of these individuals may be too young to develop glaucomatous signs at the time of examination, but have potential for developing POAG later on in life.

Because this is a cross-sectional study, it has important limitations. Exposure and disease are assessed at the same point in time, making it impossible to determine whether exposure preceded or followed the occurrence of disease. Further studies are required to assess possible associations suggested by data. Cohort or longitudinal studies are ideal as subjects are followed for a specific period of time for the occurrence of disease in each exposure group, allowing assessment of the temporal relationship between exposure and the disease

and the development of multiple outcomes from a single exposure. However, this would pose the problems of the expense of following a large group of people for a long period of time and the difficulty of retaining people in the study to minimise losses to follow-up. This would ensure the validity of study.

The strengths of the present study are the objective examination and grading of POAG, relative large sample size and random distribution of glaucoma patients throughout Tasmania. Selection biases were minimised by identifying patients from the same general ophthalmology practices-based cohort rather than referral centres or specialty clinics. Thorough data collection was possible with a relatively high participation rate (77.1% after application of exclusion criteria), which did not differ markedly between the familial and sporadic glaucoma groups. Ascertainment of probands and relatives was high. Interview bias was reduced because the study personnel were unaware of the study hypotheses and interviewers followed standard forms and procedures. Each feature of glaucoma was assessed separately in a blinded fashion to avoid biased diagnosis. Differences in technicians administering visual fields were inevitable. However, misclassification was unlikely using the standardised GIST scoring system. Ascertainment and self-selection biases may be present as the study was patient-based. Reporting of family history may be subject to recall bias, severity of glaucoma and the under-diagnosis of glaucoma in the community. However, this was minimised by comparing the genealogic information with the objective examination findings of extended pedigrees rather than relying on history data. Investigation using laboratory identification of mutations in the GLC1A gene eliminated the problems associated with family history recall in an extension study of a chosen pedigree in chapter VI.

CHAPTER XVI

CONCLUSIONS, IMPLICATIONS & RECOMMENDATIONS FOR FUTURE RESEARCH AND PRACTICES

"This is not the end. It is not even the beginning of the end. But it is, perhaps the end of the beginning"

(Winston Churchill)

XVI.I *CONCLUSIONS*

Glaucoma Characteristics – Family History, Age and Gender

In this study, 59.6% of all glaucoma subjects had a positive family history of POAG based on objective examinations and the GIST score system together with genealogic data.

There was no significant difference in the distribution of gender between the familial and sporadic glaucoma groups (OR 1.053; 95% CI 0.819 – 1.353), presumably explained by the autosomal inheritance of familial POAG.

There were more females (43.1%) in the total glaucoma group compared to the 'control' unaffected group (40.7%), but the difference was insufficient to reach statistical significance (OR 1.102; 95% CI 0.925 – 1.313). The implications of this are limited by the definition of the unaffected 'control' group, whereby some individuals classified as normal at time of examination may still potentially develop glaucoma at a later date. In addition, there are proportionately more females with increasing age and the average age of the females in the glaucoma group was 18 years older than the average age of the females in the unaffected group.

In both the familial ($p<0.001$) and sporadic ($p<0.001$) glaucoma groups, there is a non-linear decline in the proportion of subjects and increasing GIST scores, suggesting the possibility of a higher mortality rate associated with higher severity of POAG, but further investigation is required for clarification.

The distribution of GIST scores in familial glaucoma was skewed towards the higher spectrum and was significantly different from that of sporadic glaucoma ($p<0.001$), suggesting the possibility of an earlier onset and/or greater severity of glaucomatous changes in the familial glaucoma group. However, this interpretation may be limited because although the GIST score correlates with severity, it is actually a score of certainty of diagnosis of glaucoma. On the other hand, there is some evidence that certain mutations of the myocilin gene, such as Thr377met and Tyr377His, are associated with an earlier age of onset of POAG, higher peak IOPs and greater likelihood of undergoing glaucoma drainage surgery (Mackey in press, Alward 1998).

Age and gender were significant confounders in comparing familial and sporadic glaucoma groups, and should be considered in all future glaucoma studies.

Clinical Risk Factors – Familial versus Sporadic Glaucoma/ GIST Scores/ Degree of Relative Distributions

Keeping in mind the drawbacks of a study limited to a specific geographical area that depends on voluntary responses from its subjects and on sampling techniques, this thesis purports that there are no significant differences between familial POAG and sporadic POAG in the nine risk factors tested (hypertension, diabetes mellitus, migraine, corticosteroid use, smoking, atherosclerosis, cold extremities, blood transfusion, thyroid disorders).

In this investigation, there was initially a weak significant difference in odds of atherosclerosis between the familial and sporadic glaucoma groups (OR 0.70; 95%CI 0.50-0.97), which is unaffected by age and gender (95%CI 0.69; 95%CI 0.49-0.95) or by other potential risk factors (OR 0.71; 95%CI 0.51-0.99). However, the significance is lost after adjusting for GIST scores differences between the two groups (OR 0.74; 95%CI 0.53-1.03). In addition, the genetic association between degrees of relative subgroups of familial glaucoma and sporadic glaucoma is not substantiated in the current study.

The distributions of the remaining eight risk factors (hypertension, diabetes mellitus, smoking, migraine, transfusion, cold extremities, thyroid disorders and ever use of corticosteroids) were not significantly different between the two glaucoma subgroups after adjusting for other risk factors, GIST scores and age and gender.

The difference for blood transfusion was not significant in the 1st, 3rd or 4th -degree relatives subgroups, but was weakly significant in the 2nd degree relative subgroup (OR 0.565; 95% CI 0.323-0.988)(p=0.045). Similarly, the difference for thyroid disorder was not significant in the 1st, 2nd or 4th degrees relative subgroups, but was significant in the 3rd degree relative subgroup (OR 0.434; 95% CI 0.221-0.851). This anomaly may be attributed to spurious aberrations yet to be identified, or may be a consequence of chance in sampling in the study.

An extension of the study highlighted that there were no significant differences (p=0.4) in the prevalence of hypertension between subjects with and without laboratory-tested mutations in the GLC1A gene in the gTas02 pedigree of the familial glaucoma group. This implies that there is insufficient evidence from the current findings to establish a significant association between hypertension and familial POAG.

Clinical Risk Factors – Familial versus Sporadic Glaucoma/ Pedigree Distributions

To the knowledge of the author, no previous study to date has examined entire glaucoma families in such detail, including both affected and unaffected family members. The sizes of individual pedigrees, however, were still too small for valid statistical comparison of the odds of risk factors in each pedigree of the familial glaucoma group versus the sporadic glaucoma group.

Clinical Risk Factors – Total Glaucoma versus 'Controls" Group

The distributions of diabetes mellitus (age-gender adjusted OR 2.67; 95%CI 1.7-4.3) and corticosteriods (OR 2.82; 95%CI 1.35-5.87) are significantly different in the total (familial and sporadic) glaucoma group compared to the unaffected 'Controls' group. In contrast, the other seven clinical risk factors (hypertension, atherosclerosis, smoking, migraine, transfusion, cold extremities and thyroid disorders) are not significantly different between the two groups. However, this must be interpreted with reservation given the younger age profile of the 'controls' group because some individuals in this group may potentially develop POAG later on in life.

XVI.II *IMPLICATIONS AND FUTURE RECOMMENDATIONS*

The suggestion of earlier onset or greater severity of disease in the familial POAG group should be further investigated or should at least be complemented with data on age at first diagnosis of glaucoma because the age of onset of glaucoma is rather insidious.

In POAG, peripheral vision loss precludes many individuals from holding a driver's licence and decreases quality of life. Blindness in advanced stages of the disease is associated with significant mortality and morbidity (Weih 2000; McCarty 2001).

Although a high proportion (97%) of people have heard of glaucoma (Attebo 1997), only half are diagnosed in the community (Mitchell 1996; Wensor, 1998). Treatment slows progression of the disease in many cases (Mao 1991; Smith 1986) and those patients who have well-controlled IOP with surgery do not seem to progress as rapidly in terms of visual field defects or optic disc pathology (McNaught et al 2000).

At present, it is recommended that patients with a positive family history of glaucoma in first-degree relatives should be screened from the age of 40 years. If the initial assessment is normal, this is followed by 2-yearly checks until the age of 50 years and then annually thereafter (Kanski, 1999). The advantage of finding a genetic association is twofold: to be

able to identify individuals at risk of POAG that is readily treated if diagnosed early and to better understand the underlying defect that causes POAG and possibly improve rationale for treatment.

If a real relation exists between atherosclerosis and familial POAG, it is likely to be modest and can be better explored by correlating with the extent of disease measured with carotid ultrasonography. However, the association is more likely to be spurious given current understanding that atherosclerosis is closely linked to cardiovascular risk factors such as hypertension, diabetes and smoking, and adjusting for these risk factors nearly eliminated the statistical significance in the relationship (OR 0.71; 95%CI 0.51-0.99). Nevertheless, there remains a need to develop strategies in glaucoma education for the community that help to target individuals with POAG risk factors who may be less likely to visit a general practitioner or ophthalmologist.

Indeed, the discovery of GLC1A allows the possibility for predictive DNA testing in family members in appropriate circumstances under strict guidelines (Mackey 1998), and this study has been an extremely worthwhile investigation even though it did not discover any significant clinical risk markers for familial POAG with current data.

Appendix A — Informed Consent

GIST Glaucoma Inheritance Study in Tasmania
a collaborative project of the
University of Tasmania,
Eye Department, Royal Hobart Hospital
Liverpool St,
HOBART Tas 7000
Ph & Fax 03) 6222 8553

University of Melbourne,
Department of Ophthalmology
Royal Victorian Eye & Ear Hospital,
32 Gisborne St,
EAST MELBOURNE Vic 3002
Ph & Fax 03) 9929 8713

CONSENT FOR DNA TESTING

Information for patients in the Glaucoma Inheritance Study (GIST).

The glaucoma inheritance study is looking for families with glaucoma to find the genes that cause glaucoma. We are inviting individuals and families who are affected with glaucoma to be involved in the study. This is at no cost to you. We wish to take a blood sample, or a mouth swab to test your DNA to see if we can find the mutations in the first gene that we have discovered that causes glaucoma. If this first gene is not affected we may use the DNA to help discover the other genes that lead to glaucoma. You are under no obligation to provide this and it may not carry any direct benefit to your glaucoma management, but it may assist us in understanding who else in your family is at risk of glaucoma.

The DNA will be tested and we may find: A change in the DNA, no change in the DNA, or be unable to find anything. You will be informed of the result of your test and be able to discuss this with us at any time.

The DNA will be stored at the Universities of Tasmania and Melbourne. The results of any scientific development will be owned by the Universities of Tasmania and Melbourne and their collaborators. You may ask to withdraw from the study at any time, without prejudice, and have your sample destroyed. We may do further studies on glaucoma at a later date and will of course inform you of your results. In our work we may find other abnormalities of the DNA and will discuss the results with you. We may also find that you are distantly related to other families that we have studied. based on the DNA findings. These results may all be published but will never identify you specifically.

We will give you a copy of this form to keep for future reference. For more details or any questions please contact Dr David Mackey on the above numbers or leave a message. If you have any questions about the ethical nature of this study you may contact Dr Rosalie Parton of the RHH ethics committee on 03)6222 8226.

Please sign this form to certify that you read and understood the Information sheet, and had explained the nature and possible outcomes from the DNA testing and your questions have been answered to your satisfaction.
I am happy to participate in the Glaucoma Inheritance Study GIST.
Name

This is a copy for your records

Continued on next page

NAME

Please tick one of the following if these apply to you:

I do not wish to participate in this study.....

Or

I am interested but unable to participate today.....

Or

I am interested but do not want a blood test.....

Or

If you are interested please complete the forms over the page.

Appendix B Patient Family History Survey

GIST Glaucoma Inheritance Study.

Your Name..... Place of Birth .....

Spouses name .....

Please answer the following to the best of your ability Leave blank if unknown

Your father's name ..... date of birth ../../... Place.....

Your father's father's name..... date of birth ../../.....Place.....

Your father's mother's full name..... date of birth. ../../.....Place .....
(and maiden name)

Your mother's name ..... date of birth... ../../.....Place .....
(and maiden name)

Your mother's father's name..... date of birth ../../..... Place .....

Your mother's mother's full name..... date of birth ../../.....Place .....
(and maiden name)

Names of your brothers and sisters .....date of birth
(First and Surnames
with married names of sisters,
please note if deceased

If insufficient space please
use the reverse of this
sheet or attach list)

Names of your children .....

Other Relatives affected with glaucoma (please note if deceased)

Name .....Relationship.....Address .....

Name .....Relationship .....Address .....

Name .....Relationship .....Address.....

Name.....Relationship .....Address .....

Name.....Relationship .....Address.....

Name.....Relationship.....Address .....

Is anyone tracing the family tree? Name and Address .....

Could you please attach or forward a copy of your family tree?
Thank you for your help with the Glaucoma Inheritance Study

Appendix C Examination Protocol

Predilation Exam:	**Right**	**Left**	**Tick when done**
Acuity:			**Hx**
			Cons
Refraction: Distance			**Field**
			Press
and/or Readers			**Dilate**
IOP:			**Blood**
			Photo
Gonioscopy:			**Letter**
Anterior Segment			

Dilated Exam: No 1	**Right**	**Left**
Cup/Disc ratio		
Disc Size (S,M,L)	◯	◯
Other Disease		
	Score R L	signature

Stereophoto or Dilated Exam: No 2	**Right**	**Left**
Cup/Disc ratio		
Disc Size (S,M,L)	◯	◯
Other Disease		
	Score R L	signature

Stereophoto or Dilated Exam: No 3	**Right**	**Left**
Cup/Disc ratio		
Disc Size (S,M,L)	◯	◯
Other Disease		
	Score R L	signature

Field Score	**Right**	**Left**
Reliability	.....	.....
Score:A,B,C,D:	.....	.....
GIST Field Score	.....	.....

Concordance between field and discs? Yes/No

Glaucoma Type,Consistent with family's Type and other comments.

Field Score..
Pressure Score
Disc Score. .. .
GIST SCORE.

GIST

Appendix D Risk Factor Survey

Glaucoma Inheritance Study GIST G /Registry Bleed

Please complete the following information to the best of your ability. If you do not know the answer please leave the question blank.

Today's Date..... / ...../. . Time .....

Surname... .. ..

Maiden Name .... GP.. . ...

First Name. .... Ophthalmologist . . ..

Address ... . Date of Birth..... / . ../. ... .

. ... .Age... ....

....Post Code.. .. . .

Phone. ....

Do you have glaucoma?.. .. . .

When was the date of Diagnosis?..... . ... Your age at Diagnosis? ....

The highest eye pressure if known?..... .. ..

Do you have a family history of glaucoma? Yes/No
Fathers side? Yes/No
Mothers side? Yes/No
Number affected

Please name your Glaucoma Medications..... ... .

Have you ever had eye surgery or laser treatments for glaucoma? . ...

What and When?..... . ...

Do you have any other eye problems?... . . . .

Have you had any other eye surgery? (What and When)..... ....

or injury to your eyes? ....

Are you on any general medications?

Please name them if possible:. . ...

Do you have high blood pressure? Yes/No

Do you smoke? Yes/No

Do you have diabetes? Yes/No

Have you ever had a blood transfusion? Yes/No Why?

Have you ever had a heart attack, stroke or any other disease with hardening of the arteries? Yes/No

Do you get cold hands or feet? Yes/No

Have you had any thyroid problems? Yes/No

Do you suffer from migraine headaches? Yes/No

Have you ever been on Cortisone or steroid medication? Yes/No
(cortisone eyedrops, nasal spray, steroid asthma spray, cortisone skin creams, steroid injections or steroid tablets)

Appendix E Protocol for Calculation of GIST Score

INTRAOCULAR PRESSURE (IOP)

Score **1 point** if –

IOP is 22 mmHg or higher in either eye at time of examination or noted by a reliable third party prior to treatment.

An **additional point** if –

IOP is 28 mmHg or higher in either eye at time of examination or noted by a reliable third party prior to treatment.

OPTIC DISC ANALYSIS

Score **APSA** – appearance precludes satisfactory assessment if the appearance of the optic nerve head is sufficiently abnormal or has, or has had, a condition which makes exclusion of glaucoma impossible. No raw score is altered.Score.

0 point – if the appearance of optic discs is normal or consistent with normal variation and not consistent with glaucoma.Score.

1 point – if either or both optic nerve heads are unlikely to be normal and the appearances are likely to be result from glaucoma, as characterised by one or two of the following:

- A focal notch in the neuroretinal rim which does not extend to the margin
- 'Drance'-type haemorrhages
- A nerve fibre layer defect of at least two vein widths within one disc diameter of the disc margin
- A cup disc ratio (CDR) of 0.7 or greater, or 0.2 difference between the two eyes

Score **2 points** – if the appearance of either or both the optic discs are not normal and the findings are considered to be highly likely to result from glaucoma, as characterised by one or more of the following:

- An acquired pit of the optic nerve
- A notch in the neuroretinal rim extending to the margin
- A CDR of 0.8 or greater, or more than 0.2 difference between the two eyes
- 'Drance'-type haemorrhages
- Nerve fibre layer defect

An **Additional point** – if the pathological appearance of the disc is considered highly typical of the phenotype. Findings include:

- Gross posterior bowing of the lamina
- Undermining of the neuroretinal rim ('bean pot' cupping)
- CDR of 0.9 or greater

VISUAL FIELDS

Test results are assessed for reliability and defects with the following criteria:

Fixation losses

<20% losses	0
>20% losses	1

False positive errors

<33%	0
>33%	1

False negative errors

<33%	0
>33%	1

Short-term fluctuations (dB)

<4	0
4-6	1
>6	2

A field is considered reliable if it has a rating of 0 or 1. All fields with a rating of 2 or more are repeated where possible, as are tests with obvious artefacts or grossly abnormal intra-test reliability criteria.

The visual field is then classified based upon the pattern deviation plot as follows:

A – Normal field. Reliable field test where all points lie within the age-corrected normal values on the pattern deviation plot.

B – Not significant for glaucoma. Reliable field test with minor depression at one or two points not considered significant for glaucoma on the pattern deviation plot, or defects of known or unknown cause not consistent with glaucoma. This is done by comparison with given values on the Humphrey Visual Field Analyser threshold 24-2 test total deviation plot for the minimum amount of depression that varies with location (**Figure IV.8**).

C – Significant and consistent with glaucoma. Reliable field test with three or more adjacent or clustered points within a hemifield or within the nasal field with significantly reduced thresholds and a pattern deviation plot consistent with glaucoma.

D – Markedly degraded and highly consistent with glaucoma. Due to extensive field loss, reliability score may be 2 or more.

Category A and B have raw score of 0.

Category C and D score 1.

In any of the three parameters, an additional point may be awarded for a feature highly consistent with and typical of the pedigree pattern. However, only one additional point is allowed per individual, giving a maximum possible raw score of 5 and maximum GIST score of one (Coote 1996).

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